Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera

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Summary

1. Targeted enrichment of conserved genomic regions (e.g. ultraconserved elements or UCEs) has emerged as a promising tool for inferring evolutionary history in many organismal groups. Because the UCE approach is still relatively new, much remains to be learned about how best to identify UCE loci and design baits to enrich them.

2. We test an updated UCE identification and bait design workflow for the insect order Hymenoptera, with a particular focus on ants. The new strategy augments a previous bait design for Hymenoptera by (i) changing the parameters by which conserved genomic regions are identified and retained, and (ii) increasing the number of genomes used for locus identification and bait design. We perform in vitro validation of the approach in ants by synthesizing an ant-specific bait set that targets UCE loci and a set of ‘legacy’ phylogenetic markers. Using this bait set, we generate new data for 84 taxa (16/17 ant subfamilies) and extract loci from an additional 17 genome-enabled taxa. We then use these data to examine UCE capture success and phylogenetic performance across ants. We also test the workability of extracting legacy markers from enriched samples and combining the data with the published datasets.

3. The updated bait design (hym-v2) contained a total of 2590-targeted UCE loci for Hymenoptera, significantly increasing the number of loci relative to the original bait set (hym-v1; 1510 loci). Across 38 genome-enabled Hymenoptera and 84 enriched samples, experiments demonstrated a high and unbiased capture success rate, with the mean locus enrichment rate being 2214 loci per sample. Phylogenomic analyses of ants produced a robust tree that included strong support for previously uncertain relationships. Complementing the UCE results, we successfully enriched legacy markers, combined the data with published Sanger datasets and generated a comprehensive ant phylogeny containing 1060 terminals.

4. Overall, the new UCE bait design strategy resulted in an enhanced bait set for genome-scale phylogenetics in ants and other Hymenoptera. Our in vitro tests demonstrate the utility of the updated design workflow, providing evidence that this approach could be applied to any organismal group with available genomic information.

Key-words: Formicidae, molecular systematics, next-generation sequencing, phylogenomics, targeted enrichment, ultraconserved elements

Introduction

Advances in sequencing technology and laboratory protocols have invigorated phylogenetic systematics (Lemmon & Lemmon 2013; McCormack et al. 2013; Yeates et al. 2016). By coupling next-generation sequencing with genomic reduction and sample multiplexing, it has become increasingly feasible to generate genome-scale datasets from hundreds of samples within a short timeframe. Among competing approaches (e.g. transcriptomics, RADseq and targeted enrichment), the targeted enrichment of conserved or ultraconserved elements (i.e. UCEs, sensu Faircloth et al. 2012) has grown rapidly in popularity, with the technique being applied to a variety of organisms, including birds (McCormack et al. 2013a), mammals (McCormack et al. 2012), fish (Faircloth et al. 2013), reptiles (Crawford et al. 2012) and, most recently, arthropods (Blaimer et al. 2015, 2016a; Faircloth et al. 2015; Branstetter et al. 2016a,b; Starrett et al. 2016). The reason for this rapid growth is due to the many positive attributes of the UCE approach: it works with suboptimally preserved specimens and/or degraded DNA (McCormack, Tsai & Faircloth 2015; Starrett et al. 2016).
To validate our new design approach in vitro, we focus on one group within Hymenoptera, the ants (Formicidae), a lineage that originated in the Cretaceous and diversified into more than 14,000 extant species (Ward 2014). We started by synthesizing a new, ant-specific bait set, in which we included baits designed from two distantly related ant genomes. To make this updated bait set ‘back compatible’ with data from the original bait set and with data from ‘legacy’ phyloge netic markers, we included new baits targeting loci present in the original (hym-v1) bait set (Faircloth et al. 2015) as well as baits targeting 16 nuclear genes that have been commonly sequenced in insects. Following bait synthesis, we performed in vitro tests of the new bait set on all but one ant subfamily and several outgroups. We then used the sequence data to evaluate (i) capture success of the new bait set, and (ii) phylogenetic performance in ants. We also (iii) demonstrate how the inclusion of exon baits made it possible to combine sequence data from enriched samples with published datasets based on ‘legacy’ markers. Overall, our study demonstrates the utility of the updated design workflow (Faircloth in press), providing evidence that this approach could be applied to any organismal group with available genomic information.

Materials and methods

UCE bait design

To identify conserved genomic regions and design enrichment baits targeting those regions we employed an updated UCE bait design workflow (described in detail in Faircloth in press). The main difference between the updated workflow and the standard UCE workflow (sensu Faircloth et al. 2012) is that instead of performing synteny-based, genome-genome alignment to identify UCEs, short reads from multiple genomes are permissively mapped to a single base genome. The new approach greatly increases the number of identified conserved regions (Faircloth in press).

We simulated random reads from the genomes of six ‘exemplar’ hymenopterans (Table S1) using ART (Huang et al. 2012); for each genome we aligned the reads to a base genome sequence of the sawfly Athalia rosae (Hymenoptera: Tenthredinidae) using STAMPY (Lunter & Goodson 2011) with ‘substitutionrate=0.05’, ‘insertsize=400’, ‘maxbasequal=93’ and four compute threads; and we converted alignments to BAM format using SAMTOOLS (Li et al. 2009). Following alignment, we used SAMTOOLS to remove unaligned reads from the BAM alignment files, and we used BEDTOOLS (Quinlan & Hall 2010) to convert BAM files to BED format, sort the resulting BED files and merge proximate (<100 bp distance) intervals. Then, we used a program (phyluce_probe_strip_masked_loci_from_set) from the PHYLUCE v1.6 package (Faircloth 2016; all programs beginning with ‘phyluce_’ in the name are available within the PHYLUCE v1.6 package) to screen the resulting interval data and remove intervals in each BED file that overlapped repeat-masked, short (<80 bp), or ambiguous segments of the A. rosae genome. The intervals that were not filtered represent conserved sequences (<5% sequence divergence) shared between the base genome and at least one of the exemplar taxa. We then used another program (phyluce_probe_sample_subsample_table) to determine which of these conserved intervals were shared among A. rosae and all of the exemplar taxa. We buffered these regions to 160 bp and extracted sequence from the A. rosae genome corresponding to the buffered

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EXON BAIT DESIGN

To enable the integration of sequence data collected using the new UCE bait set with data generated by targeted PCR and Sanger sequencing, we designed baits to enrich 16 exons from 12 commonly sequenced nuclear genes in ants (Table S2; AbdA, Antp, ArgK, CAD, EF1α-F1, EF1α-F2, LwRh, NaK, POLD1, Top1, Ubx, Wg). We began the design process by aligning exon sequences from four, distinctly related ant species (Martiatis heureka, Leptogenys diminuta, Cercapachys jacobsoni, Myrmica tahoensis) to one another, and for each exon and ant species, we generated 120 bp baits evenly tiled across the aligned exon sequence, resulting in a tiling density of ~2x (baits overlapped by ~60 bp). We designed baits from multiple ant species in order to introduce variability into the individual baits and increase enrichment success of each exon across all ant species. We added the resulting 452 exon baits to the ant-specific hym-v2 bait set, described above, and we had the baits synthesized by MYcroarray (MYcroarray, Ann Arbor, MI, www.mycroarray.com).

TAXON SELECTION FOR ANT PHYLOGENOMICS

We selected a total of 101 taxa for inclusion in our phylogenomic study, generating new sequence data for 84 taxa (Table S3) and extracting data from available genomes for 17 taxa (Table S1). We selected taxa to test the ability of the new bait set to evenly enrich UCE loci across major ant lineages and outgroups. We also wanted to investigate phylogenetic relationships among ant subfamilies and tribes within the subfamily Myrmicinae using phylogenomic data. Among ants, we included representatives of all subfamilies, except for the extremely rare Martialinae. Outside of ants, we included eight species from five groups within the stinging wasps (Aculeata): Vespidae, Pompilidae, Tiphidiidae, Sphecidae and Anthophila (bees).

WET LAB PROTOCOL

The protocol for capturing and sequencing UCE loci closely followed the methods reported in Faircloth et al. (2015), but is described in detail in Appendix S2.

EXTRACTION OF UCE LOCI FROM GENOME-ENABLED TAXA

To examine the presence of the newly identified UCE loci across a diverse set of hymenopteran species, we used in silico methods (see Faircloth 2016) to identify and extract the updated set of UCE loci from 38 hymenopteran genomes, including 13 ant genomes (Table S1). We obtained each genome assembly from NCBI, the Hymenoptera Genome Database (Munoz-Torres et al. 2011), or directly from authors (Johnson et al. 2013). To extract the UCE loci, we aligned our UCE bait sequences to each genome using a wrapper (phyluce_probe_run_multiple_lastzs_sqlite) around LASTZ with a less conservative setting of 80% for the ‘coverage’ and ‘identity’ parameters. We then used another script (phyluce_probe_slice_sequence_from_genomes) to slice out sequence corresponding to each UCE locus identified, along with 700 bp DNA flanking either side of the buffered UCE region (160 bp). After slicing, we used a script (phyluce_assembly_match_contigs_to_baits) to determine the detection or non-detection of UCE loci across lineages and to combine the results with those from sequenced taxa.

UCE DATA PROCESSING AND MATRIX GENERATION

The sequencing facility demultiplexed and converted raw data from BCL to FASTQ format. Using the FASTQ files, we cleaned and trimmed raw reads using ILLUMIPROCESSOR (Faircloth 2013), which is a wrapper around TRIMMOMATIC (Lohse et al. 2012; Del Fabbro et al. 2013), and we assembled reads de novo using a wrapper (phyluce_assemble_assemblo_trinity) around TRINITY v2013-02-25 (Grabherr et al. 2011). After assembly, we used a program (phyluce_assembly_match_contigs_to_baits) to identify individual UCE loci from the bulk of assembled contigs and to remove paralogs. We ran the script with default parameters and compared results using the following bait files as input (i) the principal hym-v2 bait file, containing bait sequences from all exemplar genomes; (ii) the ant-specific hym-v2 bait file, containing bait sequences from the ant genomes only and (iii) the hym-v1 bait file. The principal hym-v2 bait file produced the highest
per/sample capture results, so we used the set of UCE contigs identified with this file for subsequent processing steps (Table 1).

After UCE locus identification, we used two scripts (phyluce_assembly_get_match_counts and phyluce_assembly_get_fastas_from_match_counts) to combine the UCE contigs from the sequenced taxa with UCE contigs from 17 genome-enabled taxa (ants plus a few closely related outgroups) into a single, monolithic FASTA file. We aligned all loci individually using a wrapper (phyluce_align_seqcap_align) around MAFFT v7.130b (Katoh et al. 2012) (data pre-partitioned by locus). For the bias filtering analyses, we attempted to reduce possible negative effects caused by base composition heterogeneity, saturation and/or low information content. We performed three different concatenated analyses: (i) we converted the matrix to RY-coding; (ii) we removed loci that produced gene-trees with low mean bootstrap support (lowest 10%), high rates of evolution (highest 10%) and high levels of GC variance among taxa. For all concatenated analyses, we used the maximum likelihood program RAxML v8 (Stamatakis 2014) and we performed a best tree plus rapid bootstrap search (‘-f a’ option) using GTR+ as the model of sequence evolution and 100 bootstrap replicates.

For the partitioning analyses, we analysed the complete Ants101T-F90 matrix three ways: (i) unpartitioned, (ii) partitioned by locus and (iii) partitioning analyses, we attempted to reduce possible negative effects caused by base composition heterogeneity, saturation and/or low information content. We performed three different concatenated analyses: (i) we converted the matrix to RY-coding; (ii) we removed loci that produced gene-trees with low mean bootstrap support (lowest 10%), high rates of evolution (highest 10%) and high levels of GC variance among taxa (highest 10%) and (iii) we removed all loci deviating significantly from base-composition homogeneity, as calculated with the program BcCoCa v1.1 (Kück & Struck 2014). We call the concatenated matrices by their name (‘Ants101T-F90-1263’) after applying the above filtering steps, and then we input only the 500 ‘best’ loci (best = highest mean gene-tree bootstrap scores) into the program ASTRAL v4.10.8 (Mirarab et al. 2014; Mirarab & Warnow 2015). We used only

**Table 1.** In silico capture results for UCEs and exons comparing the use of different bait set files. The files were used as input in the **phyluce v1.6 program** phyluce_assembly_match_contigs_to_probes. For UCE capture we compared the hym-v1 bait file, the principal hym-v2 bait file and the ant-specific hym-v2 bait file. For exon capture we compared the use of the synthesized bait sequences grouped by exon, the same grouped by gene, a set of complete exon sequences for 40 ant taxa, and the same but with only one exon per gene included (‘gene2’ in table). Note that we performed all in vitro enrichments using the ant-specific hym-v2 bait set, which includes 452 baits targeting 12 legacy phylogenetic markers.

<table>
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<th></th>
<th></th>
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<td></td>
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<td>Range</td>
<td>95 CI</td>
<td>Mean</td>
<td>Range</td>
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<td>95 CI</td>
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<td>10-6</td>
<td>879</td>
<td>751–1002</td>
<td>9-8</td>
<td>772</td>
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<td>1445–1661</td>
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<td>38-3</td>
<td>2249</td>
<td>1933–2365</td>
<td>18-6</td>
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<td>103-4</td>
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<td>2241</td>
<td>1931–2357</td>
<td>18-6</td>
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<td>6-10</td>
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<tr>
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<td>5-12</td>
<td>0-3</td>
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<td>5-12</td>
<td>0-3</td>
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<td>5-9</td>
<td>1-6</td>
<td>7-0</td>
<td>5-9</td>
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</tbody>
</table>

**Table 2.** Selected statistics for various alignment sets analysed in this study. The ‘Matrix name’ provides information on the filtering level for taxon occupancy (‘F’). The ‘Ants101T-F90-1238’ alignment set was filtered to remove uninformative loci, loci with high rates of evolution, and loci with high levels of GC variance among taxa. The ‘Ants101T-F90-1263’ alignment set was filtered to remove loci exhibiting significant base composition heterogeneity among taxa.

<table>
<thead>
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<th>Matrix name</th>
<th>Loci</th>
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<th>x locus length (bp)</th>
<th>Locus range (bp)</th>
<th>Locus 95 CI (bp)</th>
<th>Inform sites (bp)</th>
<th>% missing data</th>
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</thead>
<tbody>
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<td>597-8</td>
<td>180–1671</td>
<td>7-7</td>
<td>883 215</td>
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<td>1 470 193</td>
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<td>180–1671</td>
<td>7-8</td>
<td>866 954</td>
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<tr>
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<td>599-6</td>
<td>180–1671</td>
<td>8-0</td>
<td>820 220</td>
<td>19-7</td>
</tr>
<tr>
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<td>1 109 810</td>
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<td>667 208</td>
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<tr>
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<td>399 701</td>
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<td>18-3</td>
<td>154 892</td>
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<td>10 947</td>
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<td>98-5</td>
<td>642 9</td>
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<tr>
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<td>Ants101T-F90-1263</td>
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<td>8-6</td>
<td>408 893</td>
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</table>

**ANT PHYLOGENOMICS USING UCE LOCI**

We conducted multiple phylogenomic analyses on the complete dataset to explore the ability of the new UCE data to resolve relationships among ant subfamilies and genera. We separate our analyses into three categories: (i) ‘partitioning analyses’, (ii) ‘bias filtering’ analyses and (iii) ‘species tree’ analyses. For all concatenated analyses, we used the maximum likelihood program RAxML v8 (Stamatakis 2014) and we performed a best tree plus rapid bootstrap search (‘-f a’ option) using GTR+ as the model of sequence evolution and 100 bootstrap replicates.

For the partitioning analyses, we analysed the complete Ants101T-F90 matrix three ways: (i) unpartitioned, (ii) partitioned by locus and (iii) partitioning using the ant cluster algorithm in PartitionFinder v1.1.1 (Lanfear et al. 2012) (data pre-partitioned by locus). For the bias filtering analyses, we attempted to reduce possible negative effects caused by base composition heterogeneity, saturation and/or low information content. We performed three different concatenated analyses: (i) we converted the matrix to RY-coding; (ii) we removed loci that produced gene-trees with low mean bootstrap support (lowest 10%), high rates of evolution (highest 10%) and high levels of GC variance among taxa (highest 10%) and (iii) we removed all loci deviating significantly from base-composition homogeneity, as calculated with the program BcCoCa v1.1 (Kück & Struck 2014). We call the concatenated matrices for analyses 2 and 3 ‘Ants101T-F90-1238’ and ‘Ants101T-F90-1263’, respectively, with the last number indicating the number of retained loci. For species tree estimation, we generated gene trees for all loci using RAxML, and then we input only the 500 ‘best’ loci (best = highest mean gene-tree bootstrap scores) into the program ASTRAL v4.10.8 (Mirarab et al. 2014; Mirarab & Warnow 2015). We used only...
the 500 best loci to reduce bias from loci with low information content (see Meiklejohn et al. 2016). We calculated species tree support by performing 100 multi-locus bootstrap replicates (Seo 2008). For additional details on phylogenomic analyses see Appendix S2.

EXON EXTRACTION & MATRIX GENERATION

The synthesis of the ant-specific hym-v2 bait set included baits targeting 16 exons from 12 commonly sequenced nuclear genes. To extract these exons from the 84 enriched taxa and to combine the data with publicly available sequences, we performed the following steps: First, we created four different ‘bait’ files for extracting the loci from the complete pool of contigs. These bait files were: (i) all of the 452 synthesized bait sequences, grouped by exon; (ii) the same as (i), but grouped by gene; (iii) complete exon sequences for 40 different ant taxa and all targeted exons (P.S. Ward, unpublished data), grouped by gene; and (iv) the same as (iii), but with only one exon included per gene (ArgK, CAD, Lw/Rh have multiple exons). Next, we tested the ability of the different bait files to extract exon sequences from the pool of contigs for each sample using a script (phyluce_assembly_match_contigs_to_baits). We ran the script using 80% for the ‘m in-coverage’ and ‘min-identity’ parameters, and we created a duplicates file using the ‘keep-duplicates’ command. This duplicates file records which loci PHYLUCE removes due to possible paralogy. After comparing results for each of the bait files, we found that bait file (iv) performed the best across all taxa and across ants (Table 1). Thus, we used this set of identified contigs for subsequent steps. After extracting the exons, we used two scripts (phyluce_assembly_get_match_counts and phyluce_assembly_get_fasta_from_match_counts) to generate a monolithic FASTA file containing all taxa and exons.

For two loci (EF1α/F1 and EF1α/F2) the above approach performed poorly because the copy variants were removed as paralogs. To add these loci to the dataset, we used the duplicates file generated by PHYLUCE to identify and manually extract the exons from the contigs file of each sample. We verified the identity of each EF1α copy using BLAST, and appended the sequences to the master FASTA file containing all taxa and exons.

In addition to the exons, we used PHYLUCE to extract sequence data for the ribosomal RNA genes 18S and 28S from off-target reads. Specifically, we downloaded sequences of 18S and 28S from GenBank (M. tahoensis: AY703495.1 and AY703562.1), and we used a script (phyluce_assembly_match_contigs_to_barcodes) to slice out matching sequence from the pool of contigs for each enriched sample. We then manually checked the extracted sequences and appended each to the monolithic FASTA file. Although the bait set we synthesized does not specifically target these loci, they are present in multiple copies in the genome and are thus more likely to be present in off-target sequences than other less abundant genes.

After extracting the exon and rDNA loci from enriched samples, we used a script (phyluce_align_seqcap_align) to separate, align (with MAFFT) and trim sequences from individual loci using default settings. We used MESQUITE v3.0.3 (Maddison & Maddison 2016) to inspect each resulting alignment, and we removed introns and sequences containing missing data. Next, we combined the exon and rDNA loci that we extracted from enriched samples with sequence data from published ant datasets. These included studies on all ants (Brady et al. 2006; Moreau & Bell 2013), and more focused studies on the ant subfamilies Dolichoderinae (Ward et al. 2010), Dorylinae (Brady et al. 2014), Myrmicinae (Ward et al. 2015), Formicinae (Blaimer et al. 2015), Ponerinae (Schmidt 2013), Amblyoponinae (Ward & Fisher 2016) and Pseudomyrmecinae (Chomicki, Ward & Renner 2015). For details on how the datasets were combined for analysis see Appendix S2.

PHYLOGENETIC ANALYSIS OF EXON DATA

To analyse the combined data matrix containing exon and rDNA loci from enriched and published samples, we pre-partitioned the matrix by gene and codon position and used PartitionFinder v1.1.1 to select the best partitioning scheme. We analysed the matrix in RAxML using a partitioned best tree plus rapid bootstrap search (GTR+Γ, 100 bootstrap replicates). To improve backbone support, we performed two additional analyses. First, we conducted a constraint analysis in RAxML (‘-f a’ and ‘-r’ options), in which we used one of the UCE topologies (result from helcluster-partitioned analysis with genome-enabled taxa pruned) as a fixed backbone. For this search we used GTR+Γ as the model of sequence evolution and performed 100 rapid bootstrap replicates. In the second analysis, we created a new matrix in which we concatenated sequence data from the enriched exon and rDNA sequences with data from the 100 ‘best’ performing UCE loci (best = highest mean gene-tree bootstrap scores). We analysed the resulting unpartitioned matrix in RAxML using GTR+CAT (selected to decrease computation time) as the model of sequence evolution and 100 bootstrap replicates. For all resulting trees, we modified the names of taxa to reflect the current state of ant nomenclature.

Results

BAIT DESIGN

We simulated an average of 2.64 M (95 CI ± 0.2 M) read pairs from exemplar hymenopteran lineages. On average, 3-0% (95 CI ± 0.8-5%) of these reads mapped to the A. rosae base genome (Table S5). After merging intervals, filtering short and masked loci, and determining presence/absence of loci across genomes, we identified 3010 conserved regions shared among A. rosae and all six exemplar lineages. We designed 5874 temporary baits targeting 2969 of these conserved regions. We aligned this temporary bait set to an average of 2197 non-duplicate conserved regions (95 CI ± 79-7%) across each exemplar genome, and after determining which loci we detected in the exemplar taxa, we output 2161 conserved loci identified among A. rosae and at least five of the exemplar genomes. We designed 27 495 baits targeting 2161 loci from the total of seven hymenopteran genomes. After filtering for duplicate and proximate loci and merging the older (hym-v1) and newer bait sets together, the final hymenopteran bait set contained 31 829 baits targeting 2590 conserved loci (948 from the hym-v1 design; 1642 from hym-v2 design). The ant-specific bait subset contained 9446 baits targeting 2524 conserved loci (893 from hym-v1 design; 1631 from hym-v2 design). In addition, we added 452 baits targeting 16 commonly sequenced exons to the ant-specific bait set, resulting in a final ant-specific bait set containing 9898 baits.

UCE ENRICHMENT RESULTS AND MATRIX STATISTICS

Across the 84 enriched samples, including four non-formicid outgroups, we captured an average of 2214 loci per taxon using...
the principal hym-v2 bait set file (Tables 1 and S6). Among ants, the average was slightly higher at 2249 loci and ranged from 1933 to 2356 loci (95 CI ± 170 loci). There was no obvious pattern of biased capture across ants. As expected, the number of captured loci was lower in the non-formicid outgroup taxa, which had a mean capture of 1514 loci (range 1445–1661 loci; 95 CI ± 198 loci). For all taxa, the average length of UCE contigs was 922 bp (Table S6; range 369–1322, 95 CI ± 330 bp) and the average coverage per contig was 39x (range 11–77x; 95 CI ± 27x). We also examined the number of loci captured using two alternative bait set files: (i) ant-specific hym-v2 baits (as opposed to the principal hym-v2 baits, as above), and (ii) the hym-v1 bait set (Faircloth et al. 2015). Using the ant-specific bait set file produced results similar to, but slightly lower than, using all baits (Tables 1 and S6; 2205 loci vs. 2214), with the mean decrease in the number of captured loci per taxon being most prominent in outgroups (38 less in outgroups vs. 7 less in ants). Using the original bait file from hym-v1, we recovered an average of 874 loci across all taxa (range 738 to 1002 loci), far lower than the new v2 bait set.

Across the 38 genome-enabled taxa, we successfully extracted a mean of 2326 equivalent UCE loci (Table S1; range 1406–2326; 95 CI ± 95.2). For ant genomes only, the number of extracted loci was higher, with 2471 equivalent UCE loci (range 2414–2508; 95 CI ± 16-6). Although the number of extracted loci was significantly lower than the average in several hymenopteran genomes, there was no obvious phylogenetic bias in the results.

After extracting UCE contigs from the set of 84 enriched taxa and 17 genome-enabled taxa, we used the set of loci that we obtained with the principal Hymenoptera bait set file for all further analyses. We selected this set of loci because it included the highest number of loci, as compared to other capture sets. We aligned and trimmed the loci and then filtered them for varying levels of taxon occupancy (25, 50, 75, 90, 95, 98, 100%). A complete set of statistics for each occupancy level is given in Table 2. The primary alignment set used for phylogenomic analysis (Ants101T-F90) was filtered to have 90% complete taxon occupancy per locus, resulting in a concatenated matrix that included 1856 loci and 1 109 810 bp of aligned sequence data.

**ANT PHYLOGENOMICS USING UCE LOCI**

Considering major ant lineages, our results (Figs 1 and S4–S10) mostly confirm relationships found in previous studies based on legacy markers (Brady et al. 2006; Moreau et al. 2006; Moreau & Bell 2013), suggesting that our UCE data are informative and reliable. Compared to previous studies, the most notable results include recovery of Leptanillinae as the sister group to all other ants (100% support in all analyses; Martiadiinae not included); Myrmicinae as the sister group to Ectatomminae + Heteroponerinae (100% support in concatenated analyses; 67% support in ASTRAL analysis); and complete resolution among the six myrmicine tribes, with Myrmicini + Pogonomyrmecini sister to [Stenamminini + [Crematogastrini + [Solenopsidini + Attini]]]. Despite our use of genome-scale data, we could not reliably resolve relationships among poneroid subfamilies, except for Apomyrmic, + Amblyoponinae. For a more detailed description of the phylogenetic results see Appendix S2.

**EXTRACTION AND ANALYSIS OF EXON DATA**

We successfully enriched, sequenced and extracted a set of 12 commonly sequenced genes (16 exons total) from all lineages. Using the custom ‘bait’ file targeting only one exon per gene, we recovered a mean of 10-1 genes from each enriched sample (Table 1), with the mean slightly lower in non-ant taxa compared to ants only (7-0 vs. 10-2 genes). After manually adding genes that had been removed by PHYLUCE, the mean number of recovered loci increased to 11-7 genes per taxon across all samples (8-3 genes for non-ants, 11-9 for ants). At the level of individual exons, we successfully recovered a mean of 15-5 exons (out of 16 total) per taxon (15-8 exons for ants, 10-4 for non-ants). For the ribosomal genes, which we skimmed from off-target reads, we successfully extracted each gene from 83 of 84 taxa. After combining all extracted data, the final dataset for enriched taxa included complete or partial sequence data for an average of 17-5 out of 18 loci (16 exons plus the two ribosomal genes) per taxon across all samples (17-7 for ants, 12 for non-ants).

Following extraction, we combined the exon + rDNA data with similar data from nine published studies on ants. After removing duplicate taxa and aligning and trimming the data, the combined data matrix included 1060 terminals, 15 nuclear genes (all loci mentioned above plus Enolase) and 11 406 bp of aligned sequence data, including 4678 informative sites. Phylogenetic results revealed that all enriched taxa were placed in sensible positions within the ant phylogeny (Figs S1–S3). For 34 of the 84 enriched samples, the combined dataset included sequence data from two conspecific samples, one from enriched data and one from published Sanger data, and in all these cases, the enriched sample was correctly placed as closely related to its conspecific duplicate. These results confirm that the extracted exon data can be reliably recovered along with the enriched UCE loci. Although we do not discuss the broader phylogenetic results, a few novel and/or interesting findings include paraphyly of Proceratium, Prolasius and Heteroponera and monophyly of Tetraponera and New World army ants.

**Discussion**

Sequence capture of conserved genomic loci (UCEs) has emerged as a revolutionary tool for efficiently investigating the evolutionary history of organisms (Faircloth et al. 2012, 2015; Blaimer et al. 2015; Branstetter et al. 2016b; Glenn & Faircloth 2016). Here, we designed and tested an enhanced UCE bait set for performing sequence capture in Hymenoptera, with a particular focus on ants.

We demonstrated that the new bait design approach improves upon the original design of Faircloth et al. (2015) in several important ways. First, by changing the workflow by
which we identified conserved loci, we increased the number of targeted loci by 1080. Second, by including a greater number of genomes in locus identification, bait design and bait synthesis, we increased the capture success of UCE loci across ants (mean 2249 loci from enriched samples) and Hymenoptera (mean 2326 loci from 38 genome-enabled taxa). In both cases, there was no obvious pattern of biased capture performance across taxa. Because of the high average capture rate, we were able to generate a phylogenomic matrix for ants that included more loci and fewer missing data (1856 loci and 17.5% missing data in the Ants101T-F90 matrix) than previously possible using the hym-v1 bait set. Third, by targeting loci from the original bait set and legacy markers, data generated with the new bait set are combinable with published datasets. The power of this feature was exemplified by our ability to combine exon data extracted from enriched taxa with data from multiple studies. In so doing, we created the most inclusive ant phylogeny to date. It is not yet known whether data from our bait set will be combinable with data from other approaches (e.g. transcriptomes), but there is likely to be some overlap because approximately 61% of the UCE loci we captured include some coding sequence (M.G. Branstetter, unpublished data).
Finally, our bait set is customizable. By designing the principal hymenopteran bait set from seven different genomes spread across Hymenoptera, researchers can use the principal bait set in its entirety to work across all Hymenoptera or they can subset the principal bait set to focus on a particular group within Hymenoptera. In our case, we synthesized an ant-specific bait set that included baits from the phylogenetically distant ants H. saltator and A. cephalotes, while excluding baits that were unnecessary to answer our research questions. As new hymenopteran genomes become available, it will also be possible to design new customized baits by aligning our baits to these genomes. The tools for performing such customization are available as part of the updated UCE design workflow (Faircloth in press; tutorial available at http://phyluce.readthedocs.io/en/latest/index.html).

It remains unclear how many loci are necessary to resolve the majority of phylogenetic relationships, but we believe that having a greater number of loci to work with is beneficial. A major component of modern phylogenomic analysis is the ability to remove, or ‘filter’, loci that appear to have undesirable qualities, such as missing data, base composition bias, or saturation (Borowiec et al. 2015; Fernández, Edgecombe & Giri-bet 2016; Meiklejohn et al. 2016). Consequently, having more loci gives researchers greater flexibility to remove potentially problematic loci, resulting in more robust analyses and results. In this study, we filtered loci for missing data, low information content and base composition heterogeneity, and we were still able to include over 1000 loci in most analyses. Also, while some loci might be problematic in all datasets, it is likely that the set of loci that need to be filtered will vary depending on taxonomic depth and focal lineage. Thus, having a broader range of loci to choose from provides greater phylogenetic power.

Our phylogenomic results indicate that the data generated by the new bait set are phylogenetically informative within ants and close relatives and have the potential to resolve previously intractable problems. Of greatest significance, we found Myrmicinae to be sister to the ectaheteromorph clade, and we intractable problems. Of greatest significance, we found Myr-
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Data accessibility

All bait set files used in this study are available from Dryad (Branstetter et al. 2017; https://doi.org/10.5061/dryad.89n87), and from FigShare (https://doi.org/10.6084/m9.figshare.4630373.v1), where we wait maintain updated improved versions. The phyluce software package is available from GitHub (https://github.com/faircloth-lab/phyluce). Raw sequence reads and UCE contig assemblies are available from the NCBI Sequence Read Archive and GenBank respectively (NCBI BioProject PRJNA360290). Additional data, including alignments, trees, UCE contigs and tables are also available on Dryad (https://doi.org/10.5061/dryad.89n87).

References


Faircloth, B.C. (2013) Illumiprocessor: A trimmomatic wrapper for parallel adapter and quality trimming. Available at: https://doi.org/10.6079/J9LL.


