Draft *Aphaenogaster* genomes expand our view of ant genome size variation across climate gradients

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ABSTRACT

Given the abundance, broad distribution, and diversity of roles that ants play in many ecosystems, they are an ideal group to serve as ecosystem indicators of climatic change. At present, only a few whole-genome sequences of ants are available (19 of $>$ 16,000 species), mostly from tropical and sub-tropical species. To address this limited sampling, we sequenced genomes of temperate-latitude species from the genus *Aphaenogaster*, a genus with important seed dispersers. In total, we sampled seven colonies of six species: *A. ashmeadi*, *A. floridana*, *A. fulva*, *A. miamiana*, *A. picea*, and *A. rudis*. The geographic ranges of these species collectively span eastern North America from southern Florida to southern Canada, which encompasses a latitudinal gradient in which many climatic variables are changing rapidly. For the six genomes, we assembled an average of 271,039 contigs into 47,337 scaffolds. The mean genome size was 370.5 Mb, ranging from 310.3 to 429.7, which is comparable to that of other sequenced ant genomes (212.8 to 396.0 Mb) and flow cytometry estimates (210.7 to 690.4 Mb). In an analysis of currently sequenced ant genomes and the new *Aphaenogaster* sequences, we found that after controlling for both spatial autocorrelation and phylogenetics ant genome size was marginally correlated with sample site climate similarity. Of all examined climate variables, minimum temperature showed the strongest correlation with genome size, with ants from locations with colder minimum temperatures having larger genomes. These results suggest that temperature extremes could be a selective force acting on ant genomes and point to the need for more extensive sequencing of ant genomes.

INTRODUCTION

Understanding how terrestrial ecosystems will respond to ongoing shifts in climatic variables, such as temperature and precipitation, will improve our ability to manage communities and mitigate impacts of climatic change. The mean global temperature is currently on track to meet or exceed that predicted by the most extreme forecasting models (Brown and Caldeira, 2017). Climatic change is also pushing local

conditions outside the boundaries of historic ranges, potentially leading to combinations of species or entire ecosystems that have no contemporary analogs (Burrows et al., 2014). As climate-driven impacts on evolutionary responses are likely to occur over contemporary time scales, with the potential for ecological and evolutionary dynamics to affect communities and ecosystem processes (Rowntree et al., 2011; Des Roches et al., 2017), there is a need for a comprehensive study of the genetic basis of species' responses to climate (Parmesan, 2006).

The biodiversity of most terrestrial systems is great enough to be intractable to study in its entirety. To deal with this, researchers often study 'indicator' species whose responses to environmental change are broadly representative of a much wider range of taxa (Siddig et al., 2016). Ants (Formicidae), in particular, are widely used as indicator taxa (Agosti et al., 2000) as they play key roles in community dynamics and ecosystem processes, including key interactions, such as seed dispersal and the movement of soil via colony construction (Del Toro et al., 2012). Ants also are responsive to changes in temperature and other climatic variables via individual responses, changes in colony structure and community assembly (Kaspari et al., 2015; Spicer et al., 2017; Diamond et al., 2017; Diamond and Chick, 2018).

Multiple studies support the perspective that a more complete knowledge of ant genetics will increase our understanding of ant responses to environmental change (Diamond et al., 2012; Nygaard and Wurm, 2015; Stanton-Geddes et al., 2016; Boomsma et al., 2017; Penick et al., 2017). Studies of ant genomes have shed light on the evolution and social organization of ants (Libbrecht et al., 2013). One promising avenue is the possibility of genome size as an adaptive trait in ants. Recent observational studies have reported biogeographic patterns in genome size in arthropod taxa, e.g. Crustacea (Hultgren et al., 2018), and patterns in insect genomes suggest that climate may constrain genome size with cold temperatures possibly selecting for larger genome sizes (Mousseau, 1997; Petrov, 2001; Alfsnes et al., 2017). Specific to ants, previous research into genome size variation using flow cytometry found that ants have small genomes relative to other insect taxa and that their genomes display large variation across subfamilies with patterns indicative of both gradual and rapid evolution in genome size (Tsutsui et al., 2008).

At present relatively few ant species have been sequenced —20 in total, of which 19 are currently available in the NCBI Genome Database (accessed Aug 8 2018, see Supplementary Materials Table 1). Of these, most are from tropical and subtropical assemblages (Fig 1), and all but five represent unique genera (the exceptions being two species of *Atta* and three of *Trachymyrmex*). No species of *Aphaenogaster*, which are abundant ants that play key roles in the dispersal of understory plant species in North America and temperate Asia, have yet been sequenced. Previous studies have also shown that *Aphaenogaster* species' ecological and physiological responses to climatic change appear to depend both on species identity and on the geographic region in which climatic change occurs (Warren and Chick,

2013; Stanton-Geddes et al., 2016).

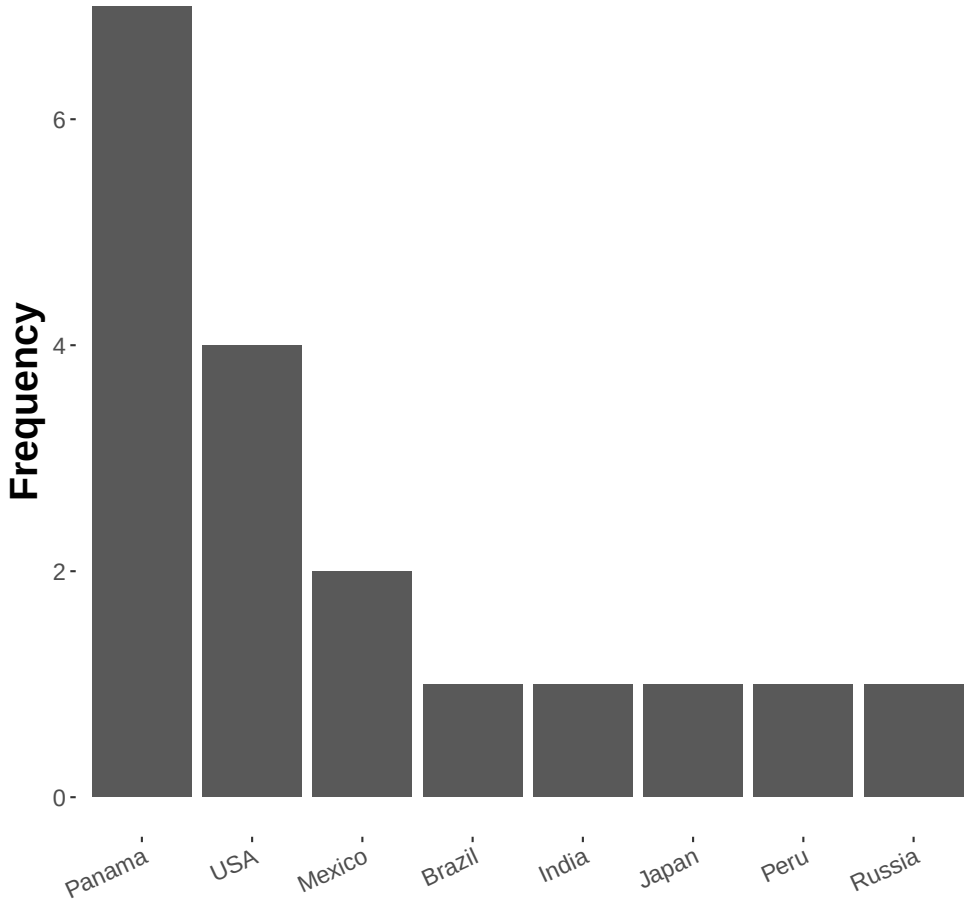

Figure 1. Number of ant (Formicidae) whole-genome sequences available in NCBI by country (accessed August 2018).

To increase the number of genomes of temperate-zone ant species, we sequenced the genomes of *Aphaenogaster* species. We conducted whole genome sequencing for six species: *A. ashmeadi*, *A. floridana*, *A. fulva*, *A. miamiana*, *A. picea* and *A. rudis*. These species were collected from across a broad biogeographic gradient spanning 10 degrees of longitude and 12 degrees of latitude. We also conducted an initial exploration of biogeographic patterns in ant genome sequences, focusing on genome size. To do this we analyzed the newly collected *Aphaenogaster* sequences together with all publicly available ant whole genome sequences. We present the newly sequenced *Aphaenogaster* genomes and investigate biogeographic (i.e. location and climate) related patterns of ant genomes using all currently sequenced ant genomes.

MATERIALS & METHODS

Sampling and Whole-genome Sequencing and Assembly

Entire colonies of the six *Aphaenogaster* species were collected by A. Nguyen and C. Penick from field sites in eastern North America (Fig 2). Ants were identified to species and voucher specimens have been deposited at the Museum of Comparative Zoology, Harvard University. Individuals from each colony were isolated from nest material and debris, weighed, placed in 50 ml Falcon centrifuge tubes, and immediately flash frozen in a -80° C freezer. Colony weights were: 794 mg (*A. ashmeadi*), 652 mg (*A. floridana*), 520 mg (*A. fulva*), 749 mg (*A. picea*), 862 mg (*A. miamiana*), 280 mg (*A. rudis* 1) and 236 mg (*A. rudis* 2).

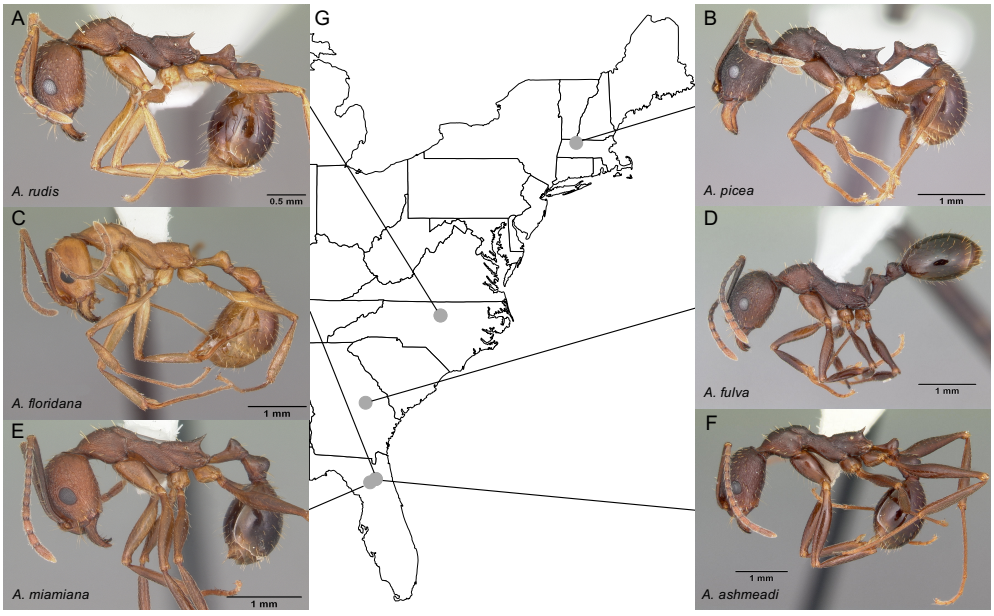

Figure 2. We sampled seven colonies representing six species of *Aphaenogaster*, including A) *A. rudis*, B) *A. picea*, C) *A. floridana*, D) *A. fulva*, E) *A. miamana* and F) *A. ashmeadi* from G) sampling locations across eastern North America (see Table 1). All photos by April Noble (available from http://www.antweb.org).

Whole colony DNA was used to have sufficient concentrations for sequencing. DNA was then extracted from each colony using methods developed previously for genomic sequencing of whole colonies of colonial mosquitos (*Anopheles* spp.) (Neafsey et al., 2010) and sequenced using an Illumina HiSeq 2500 at the Broad Institute (Cambridge, MA, USA). A combination of fragment and jump sequences were used to generate higher quality, long sequence reads.

Raw sequences were processed to remove chimeric and contaminant sequences, screened for contaminants by BLAST searches (using *blastn*) to identify sequences with likely matches to non-target species (primarily *Wolbachia* and *Mycoplasma*), and assembled using ALLPATHS-LG (version r48559) (Gnerre et al., 2011). Additional assembly processing using PILON (version 1.13) (Walker et al., 2014) was

applied to reduce base-call errors and gaps in coverage. On average, across all seven genomes, PILON reduced coverage gaps by 3.1% or 3.9 Mb. GAEMR (http://www.broadinstitute.org/software/gaemr/) software produced summary statistics of the final assembled genomes. Once assembled, repeat regions in the *Aphaenogaster* genomes were deteced and masked using *Repeatmasker* (version 4.0.5 Institute for Systems Biology).

	Lat	Lon	Tmin (C)	Tmax (C)	Precip (mm)
Aphaenogaster ashmeadi	29.79	-82.03	5.80	32.70	1314
Aphaenogaster floridana	29.79	-82.03	5.80	32.70	1314
Aphaenogaster fulva	32.69	-82.51	1.30	33.30	1155
Aphaenogaster miamiana	29.66	-82.30	5.90	32.80	1322
Aphaenogaster picea	42.60	-72.58	-12.40	28.30	1122
Aphaenogaster rudis1	36.02	-78.98	-2.70	31.50	1164
Aphaenogaster rudis2	36.02	-78.98	-2.70	31.50	1164

Table 1. Climate variables for colony sample sites. Climate are 30 year normal values (1970-2000) for minimum temperature of the coldest month (Tmin), maximum temperature of the warmest month (Tmax) and total precipitation (Precip) from the WorldClim database accessed on 08 August 2018.

Analysis of Genomes along Climate Gradients

After masking repeat regions, we applied MASH distance (Ondov et al., 2016) to measure pairwise dissimilarity of genomic sequences. The MASH method extends a data compression and dimensionality-reduction algorithm to generate estimates of sequence similarity with low computational overhead. Briefly, the pairs of genomic sequences were pre-processed into sets of k-mers of size 21 with the size of the non-redundant hashes retained set to 1,000. These settings have been demonstrated to provide good representation of genomic similarity with minimal computational costs (Ondov et al., 2016). These sets were then used to estimate the Jaccard similarity coefficient (the ratio of shared k-mers to total k-mers) of subsampled k-mer pairs of genomes. This unbiased estimate of the Jaccard similarity (J) was then used to calculate the dissimilarity of the two genomes (D) as $D = 1 - J$. All Jaccard similarity estimates had p-values less than 10^{-14}, which is below the recommended 10^{-3} probability of observing values of J due to chance.

We used multivariate correlation analyses to examine biogeographic patterns of ant genomes. Mantel tests of multivariate correlation of distance matrices were used to examine correlations among ant genomes and climate variables. Specifically, we used directional ($H_\circ$: Mantel $r \leq 0$) partial mantel tests, which calculate the correlation between two distance matrices while controlling for the covariance with other matrices (Goslee and Urban, 2007). First, we examined the correlations between genomic similarity (MASH distance), whole-genome size similarity (Euclidean distance of assembly size in total base pairs) and climate variables (also using Euclidean distance). Via partial Mantel tests, we were able to isolate the correlation between genome size and climate by controlling for spatial autocorrelation and potential

phylogenetic patterns by including geodesic and MASH distances as terms.

We obtained previously sequenced ant whole genome and climate data from a publicly available databases. Whole genome sequences for ants were obtained from the NCBI Genome database (accessed August 2018, see Supplementary Materials Table 1). Climatic variables for each sampling location was obtained from the WorldClim database (version 2.0) at a 2.5 arc minute spatial resolution from the years 1970 to 2000 (Fick and Hijmans, 2017). Although used in the previous analyses of ant genomes, two species, (*W. auropunctata* and *M. pharaonis*), which did not have published location information, were excluded from biogeographic analyses.

Using a permutational multivariate analysis of variance (PerMANOVA) procedure, we parsed the individual variables that were correlated with both genome size and MASH similarity. PerMANOVA is a flexible multivariate analog of ANOVA that permits the use of a wider set of similarity metrics to be used for the response matrix (Anderson, 2001), such as the MASH distance. We ran a total of 10,000 permutations of the original distance matrices for each statistical permutation procedure. We chose a subset of all possible climate variables available via WorldClim for this analysis. A visual inspection of the sampled climate variable correlations indicated that the primary climate variables, mean annual temperature (MAT), minimum temperature of the coldest month (Tmin), maximum temperature of the hottest month (Tmax), annual precipitation (PA) and precipitation seasonality (PS), represented the majority of climate variation (Fig 3). Based on this, we only included these variables, along with latitude and longitude coordinates, as factors in the PerMANOVAs.

It is important to note that we are using assembly size as an indicator of genome size. As genome size estimates are generally used to set assembly size targets for whole genome sequencing efforts (Hare and Johnston, 2011), we expect there to be a high degree of correlation between assembly size and genome size. Also, as a test of the potential relationship between assembly size and true genome size, we examined the correlation between the average assembly sizes of ant genera that overlapped with flow cytometry estimates of those published in Tsutsui et al. (2008). We found a marginally significant correlation assembly size and flow cytometry at the genus level (Spearman's $\rho = 0.57$, *p-value* = 0.076), which supports the use of assembly size as a useful indicator of genome size.

Data, Computation and Statistics

The raw and assembled genome sequences are currently archived at Harvard Forest (Petersham, MA, USA) and in NCBI's genome database (Genome Accessions NJRK00000000-NJRQ00000000 and BioSample Accessions SAMN06892346-SAMN06892352). Genomic distance (MASH) computations were run on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University. All analyses were conducted in **R** (R Core Team, 2017). Analytical scripts for the

project have been versioned and archived (DOI: 10.5281/zenodo.1341982) and are available online at https://zenodo.org/record/1341982. We used the *vegan* (Oksanen et al., 2016) and *ecodist* (Goslee and Urban, 2007) packages in R for multivariate analyses.

RESULTS

Genome Quality and Composition

DNA extractions yielded substantial amounts of high quality DNA with concentrations and quality scores ranging from 3.45–5.39 ngμL^{-1} and 4.05–4.27 ngμL^{-1}, respectively. All genome assemblies displayed good coverage, with an average of 70% across all genomes (Table 2). Across all species, the length of the shortest contig at 50% of the genome (i.e. N50) was 18,864 bases; average assembly GC content was 38.18%; and average genome size was 370.45 Mb. Using GAEMR's BLAST feature to conduct a search of the contigs against the NCBI's nucleotide sequence database, we discovered that 38.98% and 22.04% of the top hits were "ant" and *Aphaenogaster*, respectively. The *Aphaenogaster* genomes compared well with other ant genome sequences. The sizes of the *Aphaenogaster* genomes were within the range of other ant genomes based on size from both flow cytometry (Tsutsui et al., 2008) and the previously sequenced ant genomes available in NCBI (Fig 4).

	A. ashmeadi	*A. floridana*	*A. fulva*	*A. miamiana*	*A. picea*	*A. rudis1*	*A. rudis2*
Total Scaffold Length (Mb)	310.33	382.86	346.13	342.64	386.04	395.41	429.70
Coverage (%)	81.46	71.88	70.70	77.40	67.47	66.49	65.59
Scaffold N50 (bp)	336807.00	439114.00	255328.00	351517.00	322984.00	300103.00	269776.00
Scaffolds	5087.00	6422.00	7031.00	6920.00	6808.00	7404.00	7665.00
Max Gap (bp)	13070.00	15108.00	12104.00	11453.00	14952.00	18586.00	24564.00
Captured Gaps	26350.00	30858.00	32881.00	28801.00	36417.00	34062.00	34313.00
Total Gap Length (Mb)	57.69	107.89	101.40	77.64	125.15	131.71	148.75
Total Contig Length (Mb)	252.64	274.96	244.73	265.00	260.90	263.70	280.95
Contig N50 (bp)	21677.00	23448.00	15753.00	20738.00	15440.00	15622.00	18941.00
Contigs	31437.00	37280.00	39912.00	35721.00	43225.00	41466.00	41978.00
Assembly GC (%)	38.27	38.03	38.39	38.21	38.32	38.25	37.88
Contaminants (%)	0.30	0.24	0.02	0.26	1.14	1.25	0.61

Table 2. Sequencing statistics for the genomes of the sequenced colonies of *Aphaenogaster*.

Using the MASH genomic distances, we observed patterns of genomic similarity that were in line with expectations of ant relatedness. Sequences formed groups that corresponded with subfamilies (Fig 5). *Aphaenogaster* clustered with other genera from the Myrmicinae and, in general, subfamily level clustering tended to follow previously observed patterns of subfamily relatedness (Bolton, 2006; Moreau, 2006; Ward, 2014). The *Aphaenogaster* sequences formed a single cluster containing only *Aphaenogaster* species and displayed intra-generic levels of genomic variance comparable to other genera s(e.g., *Trachymyrmex* spp.). The separation of the two *A. rudis* species was initially surprising, as these two samples were collected at the same site (Duke Forest, NC USA) and were identified as the same species based on their morphological characteristics (Ellison, 2012; DeMarco and Cognato, 2016). However, two recent studies of targeted gene regions have demonstrated the polyphyletic nature of *Aphaenogaster rudis*.

One study of the evolution of the subfamily Myrmicinae observed that the genus as a whole could be split into at least four different lineages (Ward et al., 2015). Another, more detailed study of the genus in North America found that multiple individuals of *A. rudis* separated out into distinct groupings, each with other species, specifically, individuals of *A. rudis* from North Carolina (USA) were observed to form distinct clusters with individuals of *A. carolinensis*, *A. miamiana*, *A. lamellidens* and *A. texana* (DeMarco and Cognato, 2016).

Biogeographic Patterns of Ant Genomes

WorldClim Variable	BIO Number
Annual Mean Temperature (MAT)	BIO1
Mean Diurnal Range (MDR)	BIO2
Isothermality (Iso)	BIO3
Temperature Seasonality (TS)	BIO4
Max Temperature of Warmest Month (Tmax)	BIO5
Min Temperature of Coldest Month (Tmin)	BIO6
Temperature Annual Range (ATR)	BIO7
Mean Temperature of Wettest Quarter (MTWeQ)	BIO8
Mean Temperature of Driest Quarter (MTDQ)	BIO9
Mean Temperature of Warmest Quarter (MTWaQ)	BIO10
Mean Temperature of Coldest Quarter (MTCQ)	BIO11
Annual Precipitation (PA)	BIO12
Precipitation of Wettest Month (PWM)	BIO13
Precipitation of Driest Month (PDM)	BIO14
Precipitation Seasonality (PS)	BIO15
Precipitation of Wettest Quarter (PWeQ)	BIO16
Precipitation of Driest Quarter (PDQ)	BIO17
Precipitation of Warmest Quarter (PWaQ)	BIO18
Precipitation of Coldest Quarter (PCQ)	BIO19

Table 3. WorldClim variables, abbreviations and numbers for the climate variables used in the analysis of size and MASH similarity of ant genomes.

After controlling for both spatial autocorrelation and potential phylogenetic patterns, we found a marginally significant, positive correlation between ant genome size similarity and climate similarity (Mantel R = 0.14, *p-value* = 0.055). Although genome size similarity and MASH genome similarity were not significantly correlated (Mantel R = 0.08, *p-value* = 0.217), we included MASH as a covariate in addition to geodesic distance because previous research indicated that genome size is associated with phylogenetic relatedness (Alfsnes et al., 2017). We found that different spatial and climatic variables were associated with the size similarity of ant genomes. Longitude but not latitude was a significant predictor of genome size (Table 4). Temperature of the coldest (Tmin) and hottest (Tmax) month and total annual precipitation (PA), were significant predictors, but neither mean annual temperature (MAT) nor precipitation seasonality (PS) were significant predictors of genome size. Overall, Tmin had the highest correlation with an R^2 of 0.23. Examining the correlation between genome size and Tmin, we

observed a negative correlation with genome size tending to increase as minimum temperature decreased (Fig 6). When the newly sequenced *Aphaenogaster* genomes were excluded from the analysis, only annual precipitation (PA) was a significant predictor of genome size similarity (see Supplementary Materials Table 2).

	df	*SS*	*MS*	*Pseudo-F*	*R2*	p-*value*
Assembly Size Similarity						
Lat	1	3360.41	3360.41	2.34	0.04	0.1433
Lon	1	9238.80	9238.80	6.43	0.11	0.0181
MAT	1	267.49	267.49	0.19	0.00	0.6767
Tmin	1	20413.36	20413.36	14.21	0.23	0.0025
Tmax	1	9081.67	9081.67	6.32	0.10	0.0217
PA	1	17564.07	17564.07	12.23	0.20	0.0034
PS	1	4368.07	4368.07	3.04	0.05	0.0978
Residuals	16	22985.41	1436.59		0.26	
Total	23	87279.28			1.00	

Table 4. PerMANOVA pseudo-F table for the analysis of the factors correlated with ant assembly size.

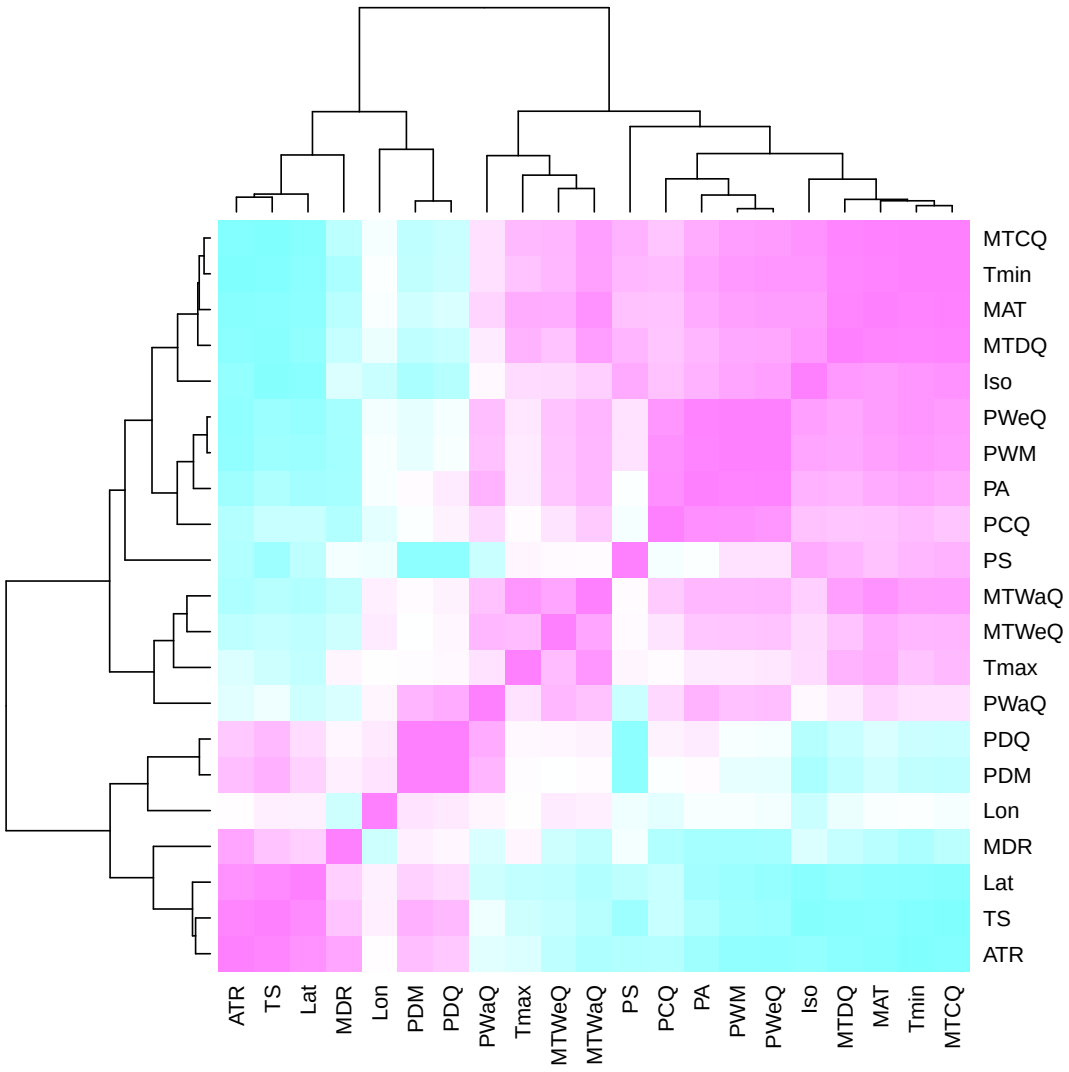

Figure 3. Heatmap of Pearson correlations among climate variables. Cells in the heatmap are colored by the correlation between the two variables that intersect at that location ranging from blue = -1 to white = 0 to pink = 1. The variables are arrayed by hierarchical clustering of the correlations, as shown by the dendrograms on the top and left side. For variable descriptions see Table 3.

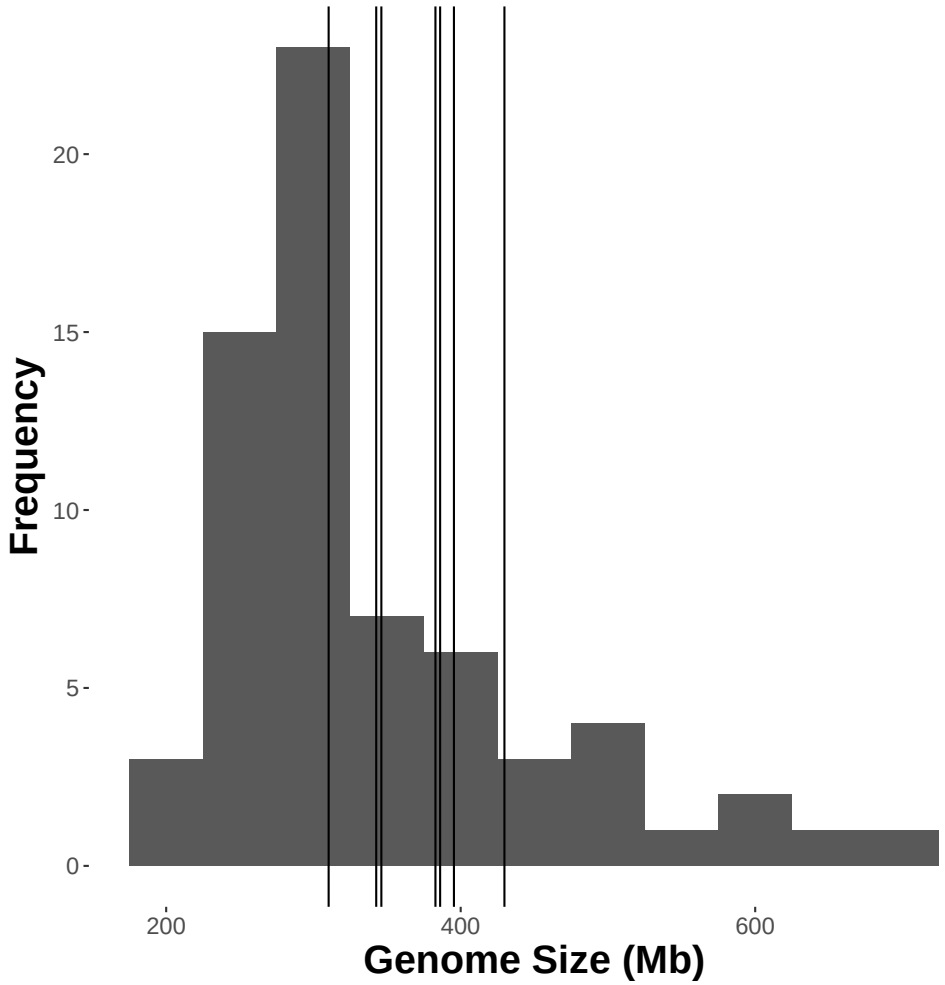

Figure 4. The size of sequenced *Aphaenogaster* genomes were within the size range of previously published observed or estimated genomes of ants. Frequency distribution of previously published genome size estimates using flow cytometry from Tsutsui et al. (2008) and those available via NCBI (accessed August 2018). Vertical lines identify the sizes of the *Aphaenogaster* assemblies (see Table 2).

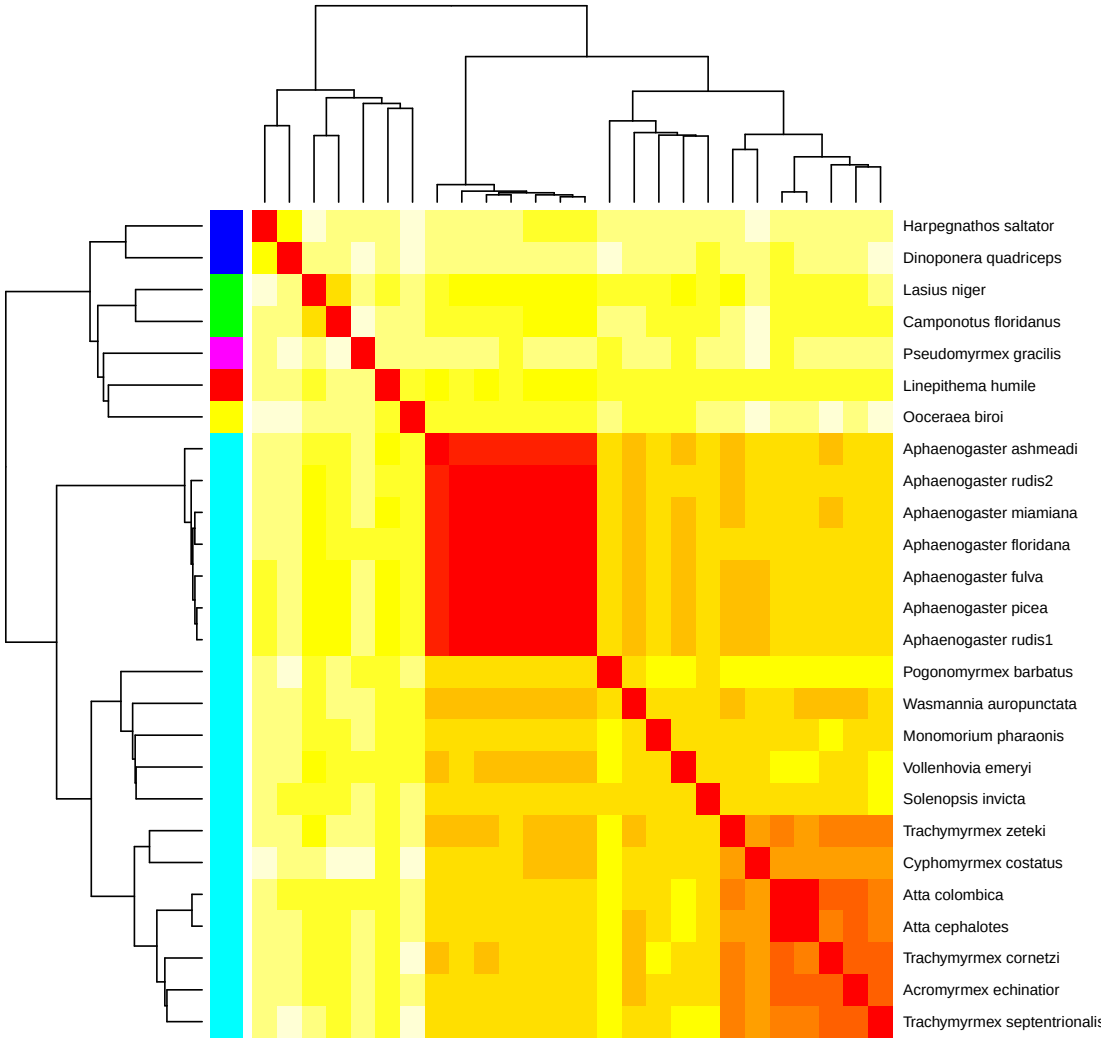

Figure 5. Heatmap of the MASH genomic distances of the *Aphaenogaster* species that we sampled together with other ant species in NCBIs. Heat colors shown in the central matrix range from high (white = 1) through moderate (orange = 0.5) to low (red = 0) genomic distance; the diagonal is entirely red because it illustrates the distance of each sequence to itself. The cladograms on the left and top show hierarchical clustering of the genomes. Colors shown to the left of the matrix indicate ant subfamilies: *Ponerinae* (dark blue), *Formicinae* (green), *Pseudomyrmecinae* (pink), *Dolichoderinae* (red), *Dorylinae* (yellow), *Myrmicinae* (light blue).

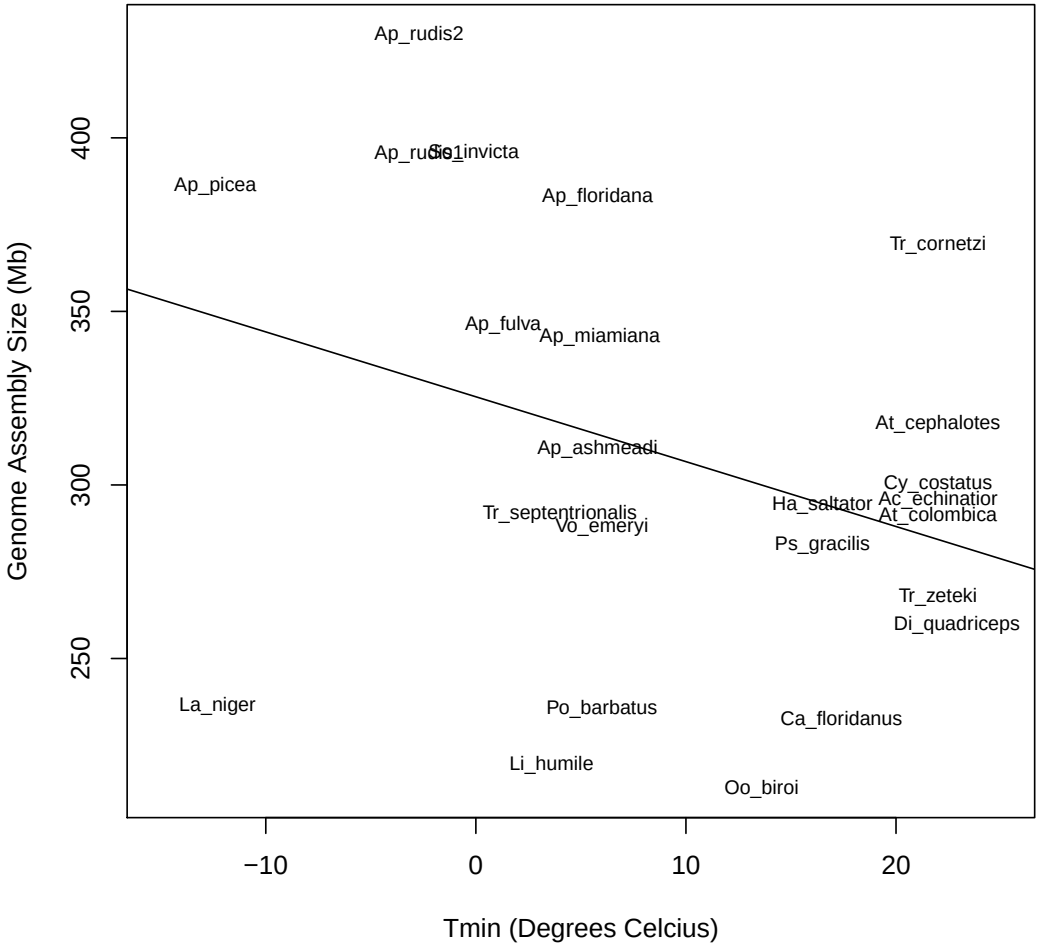

Figure 6. Bivariate plot showing the correlation between ant assembly size and minimum temperature of the coldest month (Tmin). Ants from locations with lower minimum temperatures tended to have larger genomes.

DISCUSSION

We have produced seven draft whole-genome sequences of six species of ants in the genus *Aphaenogaster*. The addition of the *Aphaenogaster* sequences increases the breadth of global ant genomic sampling, as these are the first whole-genomes from a previously un-sequenced genus, adding to the sequences of the diverse "formicoid" clade, which contains 90% of all extant ant species (Ward, 2014). Our genomic sequences were comparable in quality to other ant and insect genomes and the patterns of genomic similarity were in line with expectations based on current ant systematics. With the addition of the new *Aphaenogaster* sequences, our initial biogeographic analysis revealed that ant genomes from more similar climates have more similarly sized genomes with minimum temperatures having the strongest correlation with genome size, which is consistent with the hypothesis that climate has been a force shaping ant genome size.

Although correlative, our genome analysis results are consistent with the hypothesis that ants from regions with more similar climates tend to have similar sized genomes. Previous studies have observed physiological and ecological responses of ants to climate gradients and shifting temperatures (Warren and Chick, 2013; Stanton-Geddes et al., 2016; Diamond et al., 2016; Nguyen et al., 2017; Helms Cahan et al., 2017; Diamond et al., 2017; Penick et al., 2017) that could act as agents of selection or as environmental filters. For example, Warren and Chick (2013) found that cold, but not warm, temperatures limited shifts in the distributions of *A. picea* and *A. rudis*. Diamond et al. (2016) reported that the rate of colonization and occupancy of nests by *Aphaenogaster* species in a five-year experimental warming study (Pelini et al., 2014) declined with temperature in the warm, southern study site (Duke Forest, NC, USA) but not in the cooler, northern study site (Harvard Forest, MA, USA). In addition to the direct impacts of climate, some studies support the importance for the indirect effects of climate via biotic interactions. For example, the distribution of the species *Atta texana* is limited by the cold-tolerance of its fungal symbiont, cultivars of the genus *Attamyces* (Mueller et al., 2011). The evolution of the ant-fungus relationship has led to reductions in some ant species ranges by cold temperatures.

Specific to temperature, we found support for increasing genome size with colder temperatures. We are cautious to offer possible mechanisms for this trend. In the general context of arthropods, genome size appears to be influenced by a complex array of selection pressures, as evidenced by the recent study by Alfsnes et al. (2017), which found that genome size patterns varied greatly among major arthropod taxa with high potential for different mechanisms affecting genome size. For example, insects displayed clear phylogenetic correlations with genome size while genome size patterns in crustaceans were nearly independent of phylogeny but strongly related to biogeographic gradients (e.g. decreasing genome size with increasing maximum observed latitude). In addition, Hultgren et al. (2018) found evidence for

increasing genome size with latitude in crustaceans but not decapods, adding another example of the potential complexity of genome size as an adaptive trait.

There is the potential for both direct and indirect selection for increased genome size in colder conditions. Hessen et al. (2010) has proposed that there is a complex set of relationships among genome size, developmental rate, cell size and body size and that selection can act at different points in this causal network. One possible direct pathway is that increased expression of gene products that deal with cold stress could lead to larger genomes via whole genome or individual gene duplications Dufresne and Jeffery (2011). Previous work with *Aphaenogaster* supports this hypothesis, as Stanton-Geddes et al. (2016) found that exposure to extreme cold induced expression of genes in the cold-climate *A. picea* more so than in *A. carolinensis* (a more southern, warm climate species). An example of a possible indirect pathway is that cold could select for increased body size to deal with heat-loss in cold conditions (Brown et al., 2004), which could lead to larger genome sizes. There is evidence of cold selecting for greater body size (i.e. Bergmann's Rule) in ants (Heinze et al., 2003; Bernadou et al., 2016), and some have hypothesized that increased body size could lead indirectly to increased genome size via increased cell size (Ryan Gregory, 2005); however, the most recent, broad analysis of genome size in ants (that we are aware of) did not find support for a relationship between ant genome size and body size after controlling for phylogenetic patterns (Tsutsui et al., 2008). Therefore, assuming that our analysis adequately controlled for phylogenetics, indirect selection on genome size via body size is not a likely explanation for our observed relationship between genome size and temperature.

It is important to keep in mind that the climate related genomic patterns observed in this study should be considered an initial view of possible biogeographic patterns in ant genomes. As the addition of the these sequences had a marked impact on the statistical results of the climate analysis (see Supplementary Materials Table 2), we expect that further sequencing work will continue to enhance our understanding of the ecological genomics of ants. Also, these findings should be tested with additional sequencing efforts, as we could not control for several potentially important intercorrelated variables. Factors such as sampling bias and sequencing methodology (e.g. 454 versus Illumina) also varied among sequencing efforts, which could have contributed to some of the observed correlations with climate. We did not attempt to control for these factors statistically due to the limitations of the current ant genome sample size. Future work should methodologically and/or statistically control for such sources of variation in ant genomes as more sequences become available to illucidate clearer patterns and resolve underlying ecological and evolutionary mechanisms.

CONCLUSION

Although we have increased the total number of sequenced ant genomes by over 30%, the total number of ant sequences analyzed here is still a relatively small sample (n = 26) of the estimated >16,000 ant species and subspecies (www.antweb.org, accessed 20 Aug 2018). Efforts such as The Global Ant Genomics Alliance (GAGA)(Boomsma et al., 2017), which aims to greatly increase the number of ant species sequenced from across the world, will provide additional resources for ecological genomics studies. Further work investigating the variation in genomic content and mapping of target coding regions from previous physiological (Nguyen et al., 2017), biochemical (Helms Cahan et al., 2017), and transcriptomic (Stanton-Geddes et al., 2016) studies of *Aphaenogaster* and other ant species will inform predictions of how these species, and the ecosystems that they inhabit, may respond to ongoing climatic change. For instance, determining the genomic factors underlying the temperature response of ant assemblages to climatic gradients (Warren and Chick, 2013; Diamond et al., 2016, 2017) could provide useful insights into the response of these important organisms to non-analog ecosystem states and idiosyncratic community responses (Bewick et al., 2014). In addition, as species distribution models have been significantly improved by the inclusion of genetic information (Ikeda et al., 2016), an ecological genetics approach that couples ant genomic and ecologically relevant data will provide a useful window into the response of many terrestrial ecosystems to a changing climate.

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SUPPLEMENTARY MATERIALS

	BioProject Accession	BioSample Accession	Lat	Lon
Acromyrmex echinatior	PRJNA62733	SAMN02953789	-79.696513	9.1164638
Atta cephalotes	PRJNA48091	SAMN02953774	-79.696513	9.1164638
Atta colombica	PRJNA343260	SAMN03982875	-79.696513	9.1164638
Camponotus floridanus	PRJNA50201	SAMN02953777	-81.5431872	24.6245746
Cyphomyrmex costatus	PRJNA343963	SAMN03982885	-79.696513	9.1164638
Dinoponera quadriceps	PRJNA301625	SAMN02869781	-79.8697222	9.4008333
Harpegnathos saltator	PRJNA50203	SAMN00016742	75.7138884	15.3172775
Lasius niger	PRJNA269328	SAMN03253098	37.6172999	55.755826
Linepithema humile	PRJNA45799	SAMN02767796	-122.0230146	37.2638324
Monomorium pharaonis	PRJDB3164	SAMD00020277	NA	NA
Ooceraea biroi	PRJNA275884	SAMN02428046	127.6809317	26.2124013
Pogonomyrmex barbatus	PRJNA45797	SAMN02953770	-100.3898876	20.5888184
Pseudomyrmex gracilis	PRJNA377720	SAMN03219222	-70.8119953	-11.7668705
Solenopsis invicta	PRJNA49629	SAMN02953778	-83.357567	33.9519347
Trachymyrmex cornetzi	PRJNA343972	SAMN03982882	-79.696513	9.1164638
Trachymyrmex septentrionalis	PRJNA343973	SAMN03982881	-84.2807329	30.4382559
Trachymyrmex zeteki	PRJNA343251	SAMN03982884	-79.696513	9.1164638
Vollenhovia emeryi	PRJDB3517	SAMD00026325	-100.3898876	20.5888184
Wasmannia auropunctata	PRJDB3443	SAMD00024919	NA	NA

Table 1. NCBI genome database accession information for the previously sequenced ant genomes and coordinates for species those species that could be obtained from the published literature.

	df	*SS*	*MS*	*Pseudo-F*	*R2*	p-*value*
Assembly Size Similarity						
Lat	1	2707.43	2707.43	2.11	0.07	0.1796
Lon	1	1759.79	1759.79	1.37	0.05	0.2693
MAT	1	118.64	118.64	0.09	0.00	0.7636
Tmin	1	3394.10	3394.10	2.65	0.09	0.1434
Tmax	1	5518.63	5518.63	4.31	0.14	0.0727
PA	1	8349.14	8349.14	6.52	0.21	0.0363
PS	1	5501.51	5501.51	4.29	0.14	0.0679
Residuals	9	11533.39	1281.49		0.30	
Total	16	38882.63			1.00	

Table 2. PerMANOVA pseudo-F table for the analysis of the factors correlated with ant genome size only including the previously sequenced NCBI ant specimens.