Expanded view of the ecological genomics of ant responses to climate change

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ABSTRACT

Ecological genomics provides a window into potential responses of organisms to environmental change. 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 regions. To address this, we sequenced the genomes of seven whole colonies of six species from the genus *Aphaenogaster*: *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 comprises 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 270 Mb, which was comparable to that of other sequenced ant genomes (212.83 to 396.03 Mb). Looking across all currently sequenced ant genomes, we found support for a relationship between genomic similarity and genome size biogeographic variables. The strongest correlations were between genomic similarity and cold temperatures and precipitation. These genomic data provide a point of departure for future work that explores genotypic and phenotypic responses of ants to climatic change.

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 that are challenging to predict accurately (Burrows et al., 2014). Also, as climate driven impacts on evolutionary responses are likely to occur over contemporary

time-scales, there is a need for a comprehensive study of the genetic basis of species' climate responses to understand and potentially predict the responses of ecosystems to climate change (Parmesan, 2006; Diamond and Chick, 2018).

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) are widely used as indicator taxa (Agosti et al., 2000) because 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 are also responsive to changes in temperature and other climatic variables via both individual responses, changes in social structure and commnity assembly (Spicer et al., 2017; Diamond et al., 2017; Diamond and Chick, 2018).

In eastern North America and temperate Asia, species of the genus *Aphaenogaster* are abundant understory ants that play key roles in the dispersal of seeds. Previous studies have shown *Aphaenogaster* species respond to climatic change, and the response of these species to climatic change appears to depend both on the species being studied and on the geographic region in which climatic change occurs. Warren and Chick (2013) found that shifts in the distribution of two *Aphaenogaster* species, *A. rudis* and *A. picea*, were determined by minimum temperatures. 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). At the same sites, the southern, more warm-adapted *A. carolinensis* displayed a greater reduction in the regulation of suites of genes in response to experimental warming than did the cold-adapted *A. picea* (Stanton-Geddes et al., 2016), suggesting a genetic component to temperature response.

In addition to ants serving as indicators of ecological impacts of climatic change, ant genetics may provide insights into the potential responses of ant assemblages. A recent study found that ant colony development is experiencing selection pressure (Penick et al., 2017) and previous work has demonstrated that phylogenetics is a factor determining the response of ant species to climatic change (Diamond et al., 2012). Also, there is evidence for temporal synchrony in major transitions of terrestrial plant communities and the diversification of ant lineages. Moreau (2006) showed that the evolution of *Aphaenogaster* was coincident with the shift from gymnosperm to angiosperm dominated forests in the early to middle Paleogene. With regard to contemporary climatic shifts, there is evidence suggesting that climate change may have strong negative impacts on female individuals of dioecious plant species that could lead to increased abundance of male individuals and reductions in seed abundance and have cascading impacts

on associates (Hultine et al., 2016). Such shifts in seed producing plant species will undoubtedly have impacts on ants, especially seed dispersing species.

Although these and other studies (see Nygaard and Wurm (2015)) support the perspective that a more complete knowledge of ant genetics will increase our understanding of ant responses to environmental change (Boomsma et al., 2017), at present relatively few ant species have been sequenced —20 in total, of which 19 are currently available in the NCBI Genome Database (accessed April 1 2018). Of these, most are from tropical and subtropical assemblages, and all but five represent unique genera (the exceptions being two species of *Atta* and three of *Trachymyrmex* (Figure 1). No species of *Aphaenogaster* have yet been sequenced.

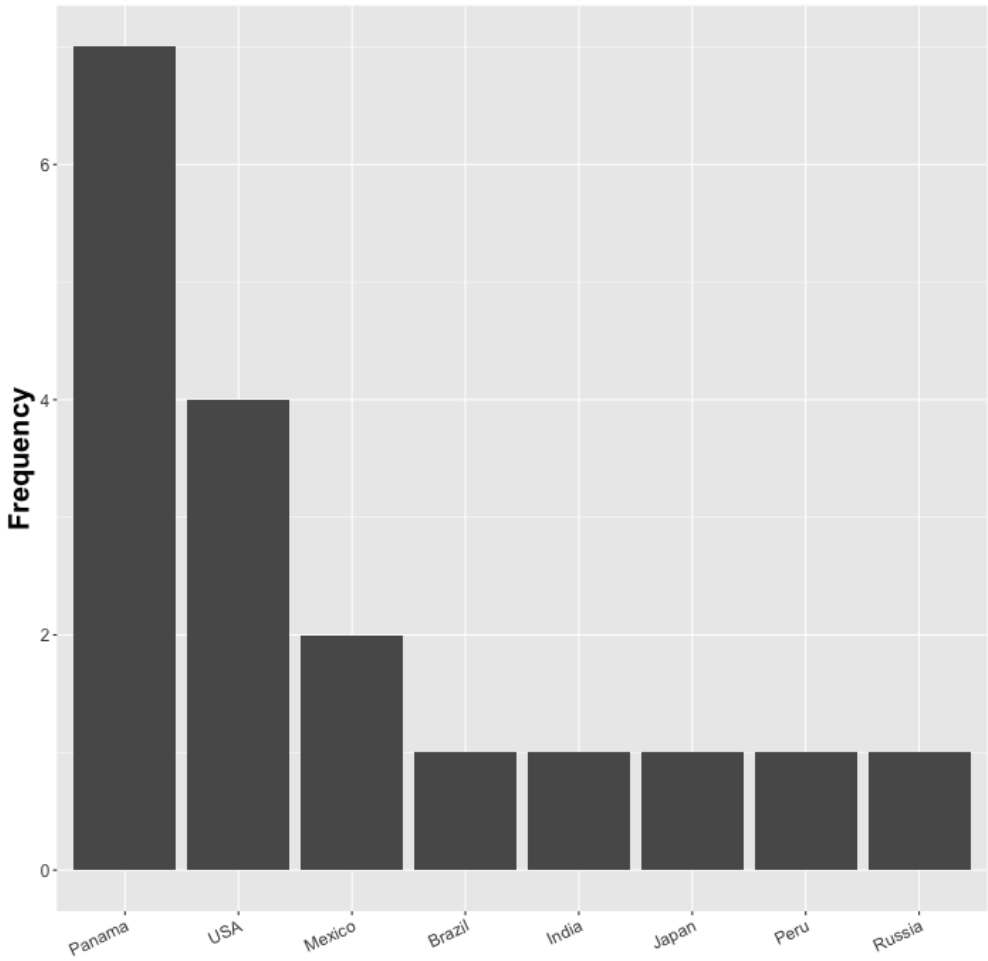

Figure 1. Number of whole-genome sequences available in NCBI (see Table for the list of NCBI accessions).

To increase the number of genomes of temperate-zone ant species for other genetic studies (e.g. short-read and target sequences or transcriptomics), we sequenced the entire genomes of six *Aphaenogaster* species from eastern North America: *A. ashmeadi*, *A. floridana*, *A. fulva*, *A. miamiana*, *A. picea* and *A.*

	BioProject Accession	BioSample Accession
Acromyrmex echinatior	PRJNA62733	SAMN02953789
Atta cephalotes	PRJNA48091	SAMN02953774
Atta colombica	PRJNA343260	SAMN03982875
Camponotus floridanus	PRJNA50201	SAMN02953777
Cyphomyrmex costatus	PRJNA343963	SAMN03982885
Dinoponera quadriceps	PRJNA301625	SAMN02869781
Harpegnathos saltator	PRJNA50203	SAMN00016742
Lasius niger	PRJNA269328	SAMN03253098
Linepithema humile	PRJNA45799	SAMN02767796
Monomorium pharaonis	PRJDB3164	SAMD00020277
Ooceraea biroi	PRJNA275884	SAMN02428046
Pogonomyrmex barbatus	PRJNA45797	SAMN02953770
Pseudomyrmex gracilis	PRJNA377720	SAMN03219222
Solenopsis invicta	PRJNA49629	SAMN02953778
Trachymyrmex cornetzi	PRJNA343972	SAMN03982882
Trachymyrmex septentrionalis	PRJNA343973	SAMN03982881
Trachymyrmex zeteki	PRJNA343251	SAMN03982884
Vollenhovia emeryi	PRJDB3517	SAMD00026325
Wasmannia auropunctata	PRJDB3443	SAMD00024919

Table 1. NCBI genome database accession information for the previously sequenced ant genomes.

rudis. These species were collected from across a broad biogeographic gradient spanning 10 degrees of longitude and 12 degrees of latitude. With the these new whole-genome sequences and the full set of publicly available ant genomes (NCBI), we test two hypotheses about the factors influencing the distribution of ant genomes. First, to test the hypothesis that climate variables shape the distribution of ant genomes, we explored the correlation between spatial and multi-decadal climate variables. If evolutionary dynamics in ants have been influenced by environmental conditions, then ant genomes from more similar conditions will have more similar genomes. Second, as previous work has demonstrated patterns in the evolutionary dynamics of ant genome size (Tsutsui et al., 2008) and empirical studies of have reported biogeographic patterns in genome size in other arthropod taxa (e.g. Crustacea (Hultgren et al., 2018)), we also tested the hypothesis that ant genome size exhibits biogeographic patterns. Because previous studies of ant genome size suggest that selection can act on genome size and that genome size is influenced by phylogeny (Tsutsui et al., 2008), we predicted that genome size similarity would also be positively correlated with environmental similarity. As higher We present the results of this sequencing effort and use of the entire set of ant genomes to test the hypotheses of biogeographic patterns in ant genome sequence similarity and size.

Whole-Genome Sequencing

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 specimens from these colonies are preserved at North Carolina State University and the Museum of Comparative Zoology at 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: 794.0 mg (*A. ashmeadi*), 652.0 mg (*A. floridana*), 520.0 mg (*A. fulva*), 749.0 mg (*A. picea*), 862.0 mg (*A. miamiana*), 280.0 mg (*A. rudis* 1) and 236.0 mg (*A. rudis* 2).

Figure 2. We sampled seven colonies representing six species of *Aphaenogaster* from across eastern North America (see Table 2). All photos by April Noble (available from http://www.antweb.org).

DNA was then extracted from each colony using methods developed previously for genomic sequencing of whole colonies of mosquitos (*Anophales* 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 in combination to generate higher quality, long sequence reads. Raw sequences were processed to remove chimeric and contaminant sequences, screened for contaminants by BLAST searches 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 used 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.

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% of fragments mapped (Table 3). Across all species, the length of the shortest contig at 50% of the genome (i.e. N50) was 18,864 bases; average assembly

	Lat	Lon	Tmin (C)	Tmax (C)	Precip (mm)
Aphaenogaster ashmeadi	29.79	-82.03	6.11	33.13	1290.40
Aphaenogaster floridana	29.79	-82.03	6.11	33.13	1290.40
Aphaenogaster fulva	32.69	-82.51	1.83	33.81	1156.81
Aphaenogaster miamiana	29.66	-82.30	5.87	32.75	1254.72
Aphaenogaster picea	42.60	-72.58	-11.11	28.12	1199.06
Aphaenogaster rudis1	36.02	-78.98	-1.82	31.60	1168.41
Aphaenogaster rudis2	36.02	-78.98	-1.82	31.60	1168.41

Table 2. Climate variables for colony sample sites. Climate are 30 year normal values (1976-2016) for January minimum temperature (Tmin), July maximum temperature (Tmax) and total precipitation (Precip) extracted from the PRISM (PRISM Climate Group, Oregon State University, USA).

GC content was 38.18%; and average genome size was 471 Mb. Using a BLAST search of the contigs and the NCBI sequence database, we found that 38.98% and 22.04% of the top hits were "ant" and *Aphaenogaster*, respectively. Both raw sequences and the assembled genomes are currently stored at Harvard Forest (Petersham, MA, USA) and NCBI's genome database (Embargoed Genome Accessions NJRK00000000-NJRQ00000000 and BioSample Accessions SAMN06892346-SAMN06892352).

	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 3. Sequencing statistics for the genomes of the sequenced colonies of *Aphaenogaster*.

The *Aphaenogaster* genomes compared well with other ant genome sequences retrieved from the NCBI database. The sizes of the *Aphaenogaster* genomes were within the range of flow-cell cytometry estimates of the genome size of a larger set of both ant and other insect species (Tsutsui et al., 2008) (Fig 3A). The size of the scaffolds were within the range recommended for gene coverage based on Efron and Tibshirani (2007) (Fig 3B).

We observed patterns in genomic composition that generally were consistent with expectations based on phylogenetic relatedness. After detecting and masking repeat regions in the *Aphaenogaster* genomes using *Repeatmasker* (version 4.0.5 Institute for Systems Biology), 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

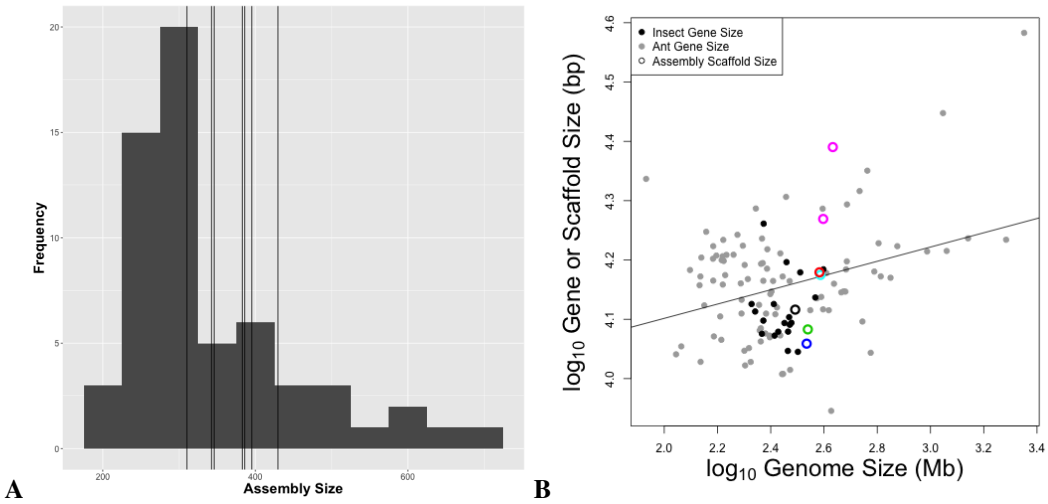

Figure 3. The size of sequenced *Aphaenogaster* genomes and the average scaffold size were within the size range of previously published observed or estimated genomes of ants and other insects. (A) Frequency distribution of previously published genome size estimates using flow cytometry; vertical lines identify the sizes of the *Aphaenogaster* assemblies. (B) A log-log plot of the whole-genome size and scaffold sizes of the *Aphaenogaster* genomes, along with those of ants and other insects (Tsutsui et al., 2008). *Aphaenogaster* genomes are indicated by open circles with the following colors: dark blue = *A. ashmeadi*, light blue = *A. floridana*, black = *A. fulva*, green = *A. miamiana*, *A. picea* = red and *A. rudis* = pink (see Table 3 for scaffold size information).

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.

Using the MASH genomic distances, we observed patterns of genomic similarity in-line with expectations from established ant phylogenetics. Sequences formed groups that corresponded with subfamily (Fig 4). *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 (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).

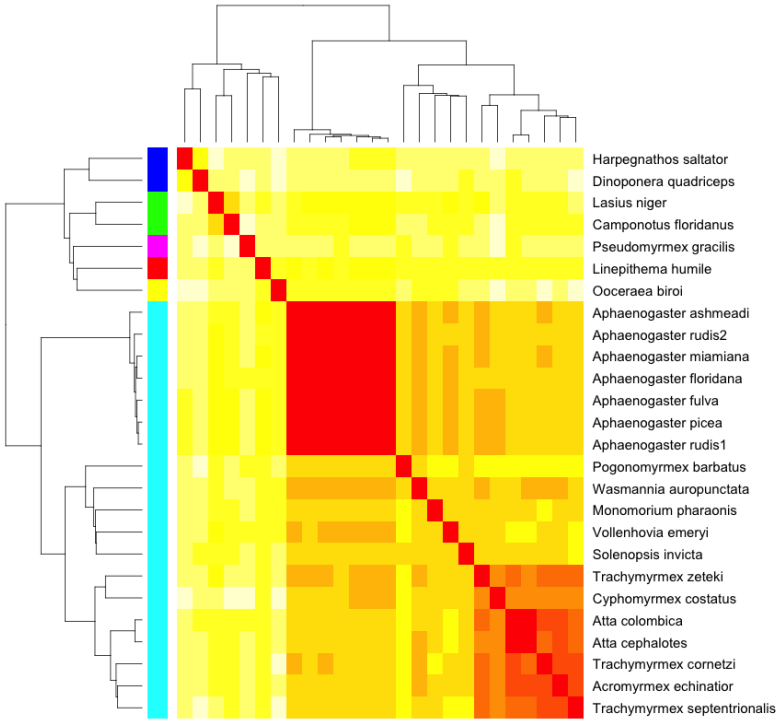

Figure 4. Heatmap of the MASH genomic distances of the *Aphaenogaster* species that we sampled together with other ant species in NCBIs. Heat colors shown in the central matrix range from high (white = 1) through moderate (orange = 0.5) to low (red = 0) genomic distance; the diagonal is entirely red 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).

Biogeographic Patterns of Genomic Structure

To examine these relationships, we conducted multivariate correlation analyses (Mantel Tests) of inter-species whole-genome size similarity using the Euclidean distance of whole-genome length (total base pairs) and genomic similarity (MASH distance) with the Euclidean distances of standardized climate variables. More specifically, we conducted directional ($H_{\circ}$: Mantel $r \leq 0$) partial mantel tests to control for spatial autocorrelation by including geodesic distance as a term (Goslee and Urban, 2007). Data for climate variables for each sampling location from the WorldClim database (version 2.0) at a 2.5 arc minute spatial resolution from the years 1970 to 2002 (Fick and Hijmans, 2017) (Table 4).

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

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 4. WorldClim variables, abbreviations and numbers for the climate variables used in the analysis of size and MASH similiarity of ant genomes.

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), annual minimum temperature, annual maximum temperature, annual precipitation and summer precipitation, represented the majority of climate variation (5). Based on this, we only included these variables, along with latitude and longitude coordinates, as factors in the PerMANOVAs.

To visualize the patterns of genomic similarity and spatio-climate variation, we used non-metric multidimensional scaling (NMDS) ordination to the MASH genomic distances using 500 iterations to produce a two-dimensional lowest stress solution for all genomes and only the *Aphaenogaster* genomes, respectively. The R^2 and stress of the final solutions were 0.80 and 15%. The geographic (latitude and longitude) and WorldClim climate variables were then correlated with both sets of MASH genomic distances (i.e. all and just *Aphaenogaster*) using a vector analyses (Oksanen et al., 2016).

We found significant global, biogeographic patterns of ant species genomes. Across all whole-genome ant sequences (both the NCBI and the newly sequenced *Aphaenogaster* species), ants from climatically similar locations tended to have similar genomes (Fig 6). We also observed that collection location climate similarity was significantly correlated with genome size similarity (Mantel r = 0.19, p-value = 0.021) and whole genome similarity (MASH distance) (Mantel r = 0.3248169, p-value = 0.001).

Both space and climate were important factors determining the size and genomic similarity of the ant genomes. Longitude but not latitude was a significant predictor of genome size (Table 5). Temperature of the coldest (Tmin) and hottest (Tmax) month and total annual precipitation (PA), were all significant predictors of genomic size similarity, but neither mean annual temperature (MAT) nor summer precipitation (PS) were significant predictors of genome size . Overall, Tmin was the strongest predictor with an R^2 of 0.23. Latitude and longitude were both correlated with MASH genome distance; and all climate variables examined were significant predictors of whole-genome similarity with Tmin (R^2 = 0.10) also being the strongest predictor. Interestingly, when the newly sequenced *Aphaenogaster* genomes were excluded from the analysis, climate was not correlated with genome size similarity (Mantel r = 0.13, *p*-value = 0.190) and only annual precipitation (PA) was a significant predictor of genome size similarity, and longitude and mean annual temperature (MAT) were significant predictors of MASH genomic similarity (Supplementary Materials Table 1).

	df	*SS*	*MS*	*Pseudo-F*	*R2*	p-*value*
Size Distance						
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	
MASH Distance						
Lat	1	0.02	0.02	3.56	0.10	0.0002
Lon	1	0.02	0.02	3.26	0.10	0.0017
MAT	1	0.01	0.01	1.97	0.06	0.0341
Tmin	1	0.02	0.02	3.30	0.10	0.0004
Tmax	1	0.01	0.01	1.89	0.06	0.0382
PA	1	0.01	0.01	1.97	0.06	0.0276
PS	1	0.01	0.01	2.14	0.06	0.0159
Residuals	16	0.11	0.01		0.47	
Total	23	0.24			1.00	

Table 5. PerMANOVA pseudo-F table for the analysis of the factors correlated with ant genome size and MASH distance.

COMPUTATION AND STATISTICS

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 are available online at the Harvard Forest Data Archive (http://harvardforest.fas.harvard.edu/harvard-forest-data-archive). We used the *vegan* (Oksanen et al.,

2016) and *ecodist* (Goslee and Urban, 2007) packages in R for the multivariate analyses.

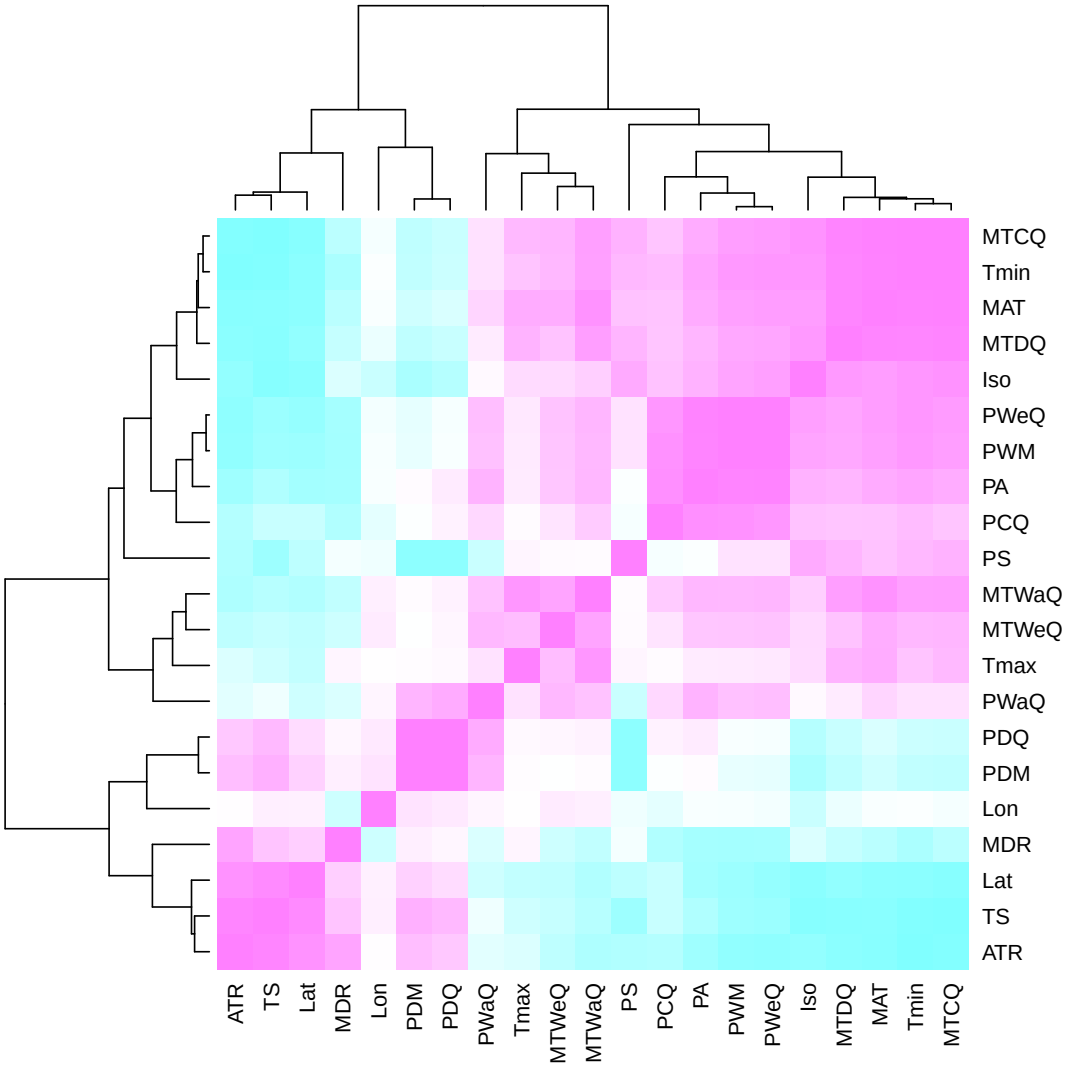

Figure 5. Heatmap of Pearson correlations among climate variables. Cells in the heatmap are colored by the correlation between the two variables that intersect at that location ranging from blue = -1 to white = 0 to pink = 1. The variables are arrayed by hierarchical clustering of the correlations, as shown by the dendrograms on the top and left side.

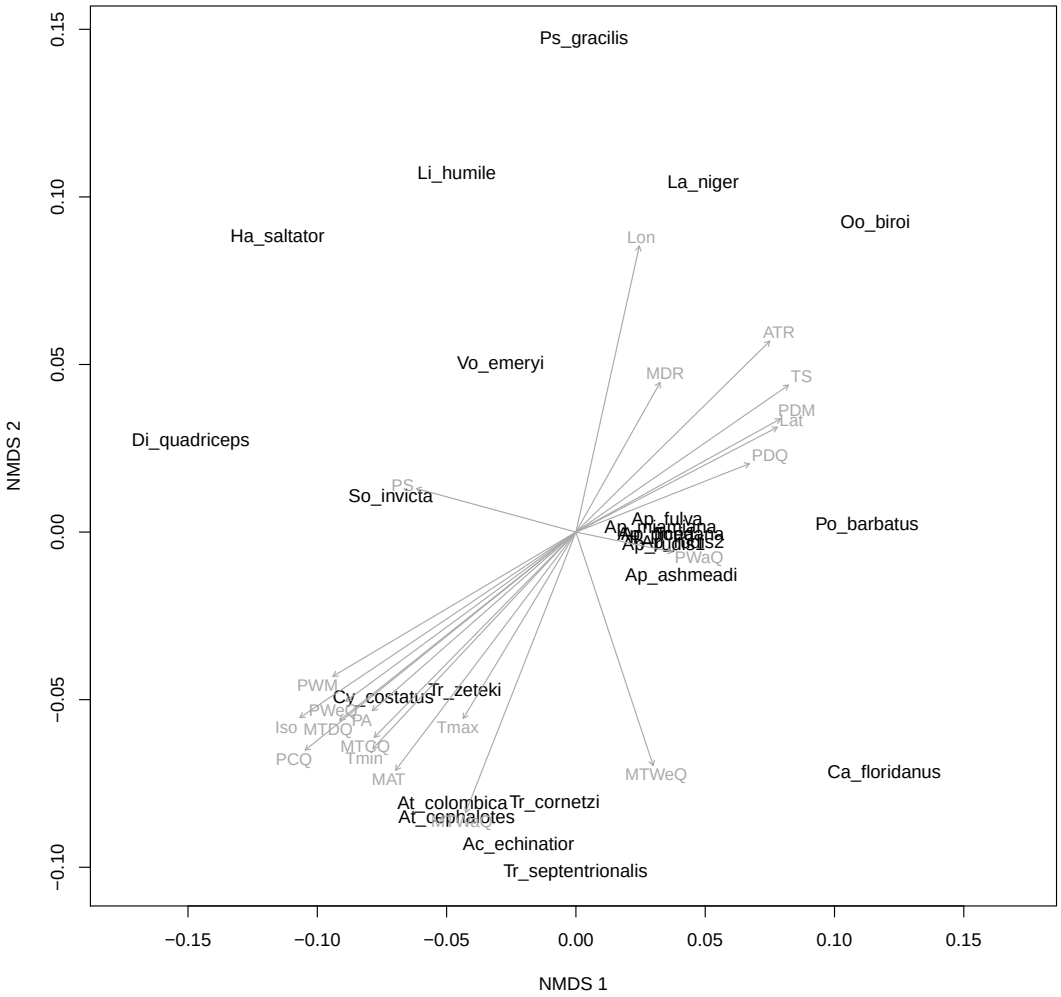

Figure 6. Plot an showing NMDS ordination of MASH genomic distance of all whole-genome ant sequences currently in NCBI and the newly sequenced *Aphaenogaster* spp. from this study. Arrows overlaid on each plot show the correlation vectors (pointing in the direction of and scaled by the correlation) between the full set of climate variables from WorldClim at the sampling locations and the genomic distance of the samples.

DISCUSSION

We have produced seven draft whole-genome sequences of six species of ants in the genus *Aphaenogaster*. 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 these sequences, we observed support for the hypotheses that genome size and similarity display spatial patterns that relate to climate. Genomic patterns across biogeographic gradients lend further weight to the importance of considering the genetic basis of climate change responses.

Our results support the overarching perspective that climate has been a force not only in shaping the distribution but also the the genetics of ant species. This is generally in-line with previous observations of physiological and ecological responses of ants to 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). We observed a strong correlation between minimum temperature and genome size and genomic similarity (MASH). Although these results are correlative, they are also consistent with previous research on the climatic determinants of ant distribution in North America. For example, (Warren and Chick, 2013) found that cold and not warm temperatures limited shifts in the distributions of two *Aphaenogaster* species (*A. picea* and *A. rudis*). With specific regard to genome size, the strong correlation with minimum temperature points to altered genome size as a potential indicator of a mechanism for adaptation to cold. The findings of a recent, broad analysis of insect genome patterns (Alfsnes et al., 2017) has demonstrated support for climatic constraints to genome size. One hypothesis being that cold temperatures could select for smaller genomes (Mousseau, 1997; Petrov, 2001; Alfsnes et al., 2017).

It is important to consider that these biogeographic patterns could be a function of other factors not examined in this study. We examined the role of both space and climate, however, we did not examine or control for phylogeny in explicitly given the small sample size of ant genomes. The genomic patterns we observed are likely to be a function of both phylogeny and ecological variation, as previous research has observed significant climate variation in insect genomes even after controlling for phylogenetic relatedness (Alfsnes et al., 2017). However, future work should disentangle the partial correlations of phylogenetics and biogeographic variation in ant genomes, once more sequences become available. In addition, interactions of ants with other organisms are likely a strong factor at play that could be a function of or interact with both space and climate. 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 lead to reductions in some ant species ranges by

cold temperatures. We observed patterns corroborating this in our analysis in the correlation between temperature variables and the clustering of similar genomes of ant species from the tribe Attini (see Fig 6).

Further work investigating the variation in genomic content and mapping of target coding regions from from previous experimental physiological (Nguyen et al., 2017), biochemical (Helms Cahan et al., 2017), and transcriptomic (Stanton-Geddes et al., 2016) work on *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 example, 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). Also, 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 likely provide a useful window into the response of a range of terrestrial ecosystems to climatic change.

CONCLUSION

The addition of the *Aphaenogaster* sequences have increased the breadth of global ant genomic sampling. 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 16 April 2018). As the addition of the *Aphaenogaster* sequences had a marked impact on the statistical results of the climate analysis, we expect that further sequencing work will continue to shift our perspective of the ecological genomics of ants. Although our analysis did include some statistical control of spatial-autocorrelation, these results are still correlative and do not eliminate other important factors that might covary with climate, such as phylogeny. Additional analytical and experimental work will be necessary to parse out a clearer understanding of the mechanisms behind these patterns. New sequencing work has been initiated by The Global Ant Genomics Alliance (Boomsma et al., 2017), which aims to greatly increase the number of ant species sequenced from across the world. These efforts will enhance our ability to resolve a clearer picture of the future impacts of global climate change.

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SUPPLEMENTARY MATERIALS

	df	*SS*	*MS*	*Pseudo-F*	*R2*	p-*value*
Size Distance						
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	
MASH Distance						
Lat	1	0.02	0.02	1.66	0.08	0.0683
Lon	1	0.02	0.02	2.07	0.10	0.0295
MAT	1	0.02	0.02	1.95	0.10	0.0332
Tmin	1	0.01	0.01	1.06	0.05	0.3679
Tmax	1	0.01	0.01	1.43	0.07	0.1483
PA	1	0.01	0.01	1.38	0.07	0.1590
PS	1	0.02	0.02	1.56	0.08	0.0871
Residuals	9	0.09	0.01		0.45	
Total	16	0.19			1.00	

Table 1. PerMANOVA pseudo-F table for the analysis of the factors correlated with ant genome size and similarity (MASH distance) only including the previously sequenced NCBI ant specimens.