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Original Article

An assemblage-level comparison of genetic diversity and population genetic structure between island and mainland ant populations

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Abstract

Island biotas provide unparalleled opportunities to examine evolutionary processes. Founder effects and bottlenecks, e.g., typically decrease genetic diversity in island populations, while selection for reduced dispersal can increase population structure. Given that support for these generalities mostly comes from single-species analyses, assemblage-level comparisons are needed to clarify how (i) colonization affects the gene pools of interacting insular organisms, and (ii) patterns of genetic differentiation vary within assemblages of organisms. Here, we use genome-wide sequence data from ultraconserved elements (UCEs) to compare the genetic diversity and population structure of mainland and island populations of nine ant species in coastal southern California. As expected, island populations (from Santa Cruz Island) had lower expected heterozygosity and Watterson's theta compared to mainland populations (from the Lompoc Valley). Island populations, however, exhibited smaller genetic distances among samples, indicating less population subdivision. Within the focal assemblage, pairwise F_{st} values revealed pronounced interspecific variation in mainland-island differentiation, which increases with gyne body size. Our results reveal population differences across an assemblage of interacting species and illuminate general patterns of insularization in ants. Compared to single-species studies, our analysis of nine conspecific population pairs from the same island-mainland system offers a powerful approach to studying fundamental evolutionary processes.

Keywords: genetic variation, population structure, natural selection, dispersal

Introduction

Island populations can offer unique insights into the relative strength of different evolutionary forces. Given the discrete nature of islands and their relatively restricted area, island populations often support reduced levels of genetic diversity compared to mainland populations (Frankham, 1997), although exceptions to this pattern exist, especially for islands that lie within the dispersal capabilities of the organisms in question (Aleixandre et al., 2013; Fernández-Mazuecos & Vargas, 2011; Francisco et al., 2016; García-Verdugo et al., 2015; Kaeuffer et al., 2007; McLaughlin et al., 2014; Patiño et al., 2017). Founder effects and bottlenecks can reduce effective population sizes on islands (England et al., 2003; Nei et al., 1975) and increase the influence of genetic drift (Motro & Thomson, 1982; Vucetich & Waite, 1999). Island area and isolation further influence the genetic structure and diversity of island populations (Frankham, 1997; Jaenike, 1973; Losos & Ricklefs, 2009) by reducing gene flow from mainland populations or from populations on other islands (Karron, 1987). Furthermore, evolution in island populations can be influenced by other species, e.g., through biotic interactions or hybridization (Lancaster et al., 2006), yet most comparisons of mainland and island populations focus on one or a few species (Dodd & Helenurm, 2002; Francisco et al., 2016; Wauters et al., 2018; Zheng et al., 2018). Given the potential for species interactions to influence evolution in island populations, assemblage-level analyses are needed to clarify how the strength of evolutionary forces varies within sets of interacting species (Gillespie, 2004).

Novel selection pressures in island environments can act in concert with founder effects and bottlenecks to influence the evolutionary trajectory of island populations. Selection for reduced dispersal ability, e.g., occurs in a variety of insular organisms including plants, birds, and arthropods (Bell et al., 2015; Gillespie et al., 2018; Hume & Martill, 2019; Kavanagh & Burns, 2014; Medeiros & Gillespie, 2011; Waters et al., 2020; Wright et al., 2016). Although the ability to disperse provides numerous ecological advantages (Bonte et al., 2014), dispersal is energy-intensive and risky (Bonte et al., 2012). Terrestrial species that colonize islands may thus trade off dispersal ability for enhanced reproductive success (Braendle et al., 2006; Gu et al., 2006). For passively dispersing organisms, selection for reduced dispersal can also result from fitness costs associated with transport off an island or beyond the bounds of narrowly distributed habitat types (Carlquist, 1966, 1974, 1980; Roff, 1986). While many studies have noted reductions in dispersal ability within island

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Table 1. For each of the nine ant species included in this study, Table 1 lists the two letter species code used within figures, the number of paired collection sites (see Figure 1), the number of SNPs and UCE sequences used in the genetic comparisons, and the pairwise F_{st} between mainland (LPC) and island (SCR) populations.

Species	Species Code	No. paired collection sites	No. SNPS	Pairwise F_{st} (LPC—SCR)	No. UCE sequences
Monomorium ergatogyna	ME	9	1164	–0.0024	1660
Prenolepis imparis	PI	10	1853	0.1089	2040
Solenopsis molesta	SM	5	1739	0.1103	2287
Crematogaster marioni	CM	6	1457	0.1403	1873
Tapinoma sessile	TS	10	1095	0.1647	1932
Pheidole hyatti	PH	9	2179	0.1565	2386
Formica moki	FM	9	2070	0.2936	2257
Dorymyrmex us-ca05	DU	10	1766	0.3183	2268
Camponotus hyatti	CH	9	2125	0.4614	2271

populations by examining the evolution of entirely flightless species (Medeiros & Gillespie, 2011; Wagner & Liebherr, 1992; Wright et al., 2016), reduced capacity for dispersal may also be evident from genetic structure (Gaston, 2003; Waters et al., 2020). Moreover, interspecific disparities in dispersal ability influence community assembly by governing the frequency of propagules arriving at a particular location (Andersen, 2008; King & Tschinkel, 2016; Livingston & Jackson, 2014), thus further influencing the evolutionary trajectories of island populations as a result of gene flow and differentiation. An assemblage-level analysis is required in order to determine the extent to which interacting species are influenced by insularization, and to clarify the effects of ecological and life-history traits on the genetic diversity and structure of island populations (Economo & Sarnat, 2012; Gillespie, 2004; Harvey et al., 2017; Losos & Ricklefs, 2009).

Ants inspired early theories and tests of island biogeography and community assembly (Cole, 1983; MacArthur & Wilson, 1967; Vepsäläinen & Pisarski, 1982; Wilson, 1961) and continue to provide insights into evolutionary processes within insular systems (Economo & Sarnat, 2012; Matos-Maraví et al., 2018; Sarnat & Moreau, 2011). Community assembly in ants can be influenced by interspecific interactions, such as competition and social parasitism (Cole, 1983; Economo & Sarnat, 2012; Vepsäläinen & Pisarski, 1982). Early colonists may experience ecological release in the absence of competitors (Cole, 1983) and interspecific disparities in colonization frequency and gene flow may influence the composition of assemblages over time (Economo & Sarnat, 2012; Wilson, 1961). Assemblage-level tests of how island populations differ from mainland populations are thus needed to clarify how colonization and establishment affect the gene pools of related and interacting island organisms (Gillespie et al., 2012, 2018).

Most ant species produce aerially dispersing, winged reproductives (Helms, 2018; Hölldobler & Wilson, 1990; Ward, 2006), and winged gynes (i.e., queens prior to mating) vary in size, timing of emergence, and colony founding strategy (Helms, 2018; Hölldobler & Wilson, 1990). In species with independent colony founding, winged gynes are responsible both for dispersal from their natal colony and for founding a new colony. An evolutionary trade-off thus exists between dispersal ability and the size of nutritional loads needed to initiate a colony (Helms, 2018; Helms & Kaspari, 2014, 2015). Increasing body size allows gynes to fly farther via powered flight (Helms, 2018), but decreases their ability to use rising air currents and to fly at higher altitudes (Dillon et al., 2006; Dudley, 2002), which may be important for long-distance and wind-assisted dispersal. Given this trade-off, gyne morphology likely influences dispersal ability, colonization ability, population genetic structure (Chapuisat et al., 1997; Pamilo et al., 1992; Sundström, 1995), and patterns of succession in community assembly (Andersen, 2008; King & Tschinkel, 2016; Livingston & Jackson, 2014), but few data exist to evaluate whether or not gyne morphology predicts gene flow and colonization ability.

In this study, we compare mainland and island populations of nine ant species (Table 1) from coastal southern California with respect to genetic diversity and population genetic structure. Using genomic data from high-throughput sequencing of ultraconserved elements (UCEs), we test the following hypotheses: (i) the island assemblage supports reduced levels of genetic diversity compared to the mainland assemblage, (ii) the island assemblage exhibits greater population genetic structure, consistent with the evolution of reduced dispersal capacity, (iii) differentiation between mainland and island populations increases with increasing gyne size because wind is the main factor that disperses gynes across large distances, and differentiation within the mainland population decreases with gyne size because powered flight is the main factor that disperses gynes across more local distances. To make these comparisons, we obtained genomic sequence data from UCEs from replicate samples of island and mainland populations. This approach exploits recent advances in the sequencing of large, orthologous sets of genetic loci that have revolutionized among-lineage comparisons of divergence and genetic diversity (Hahn, 2019; Stiller et al., 2020; Winker et al., 2018). A targeted bait set of ultraconserved elements for ants (Branstetter et al., 2017a), e.g., has helped to resolve the ant tree of life (Blaimer et al., 2018; Branstetter & Longino, 2019; Romiguier et al., 2022; Williams et al., 2020). The use of high-throughput sequencing data, when applied to assemblage-level comparisons of population genetic diversity and structure, can provide powerful insights into the mechanisms driving population differentiation (Edwards et al., 2022). Sequence data from UCEs, e.g., were used to clarify that upland forest bird species exhibit higher genetic diversity and population differentiation in comparison to closely related taxa that occur in floodplains (Harvey et al., 2017).

Santa Cruz Island is the largest of the eight California Channel Islands and supports 35 species of native ants, none of which are considered island endemics. Here, we focus on

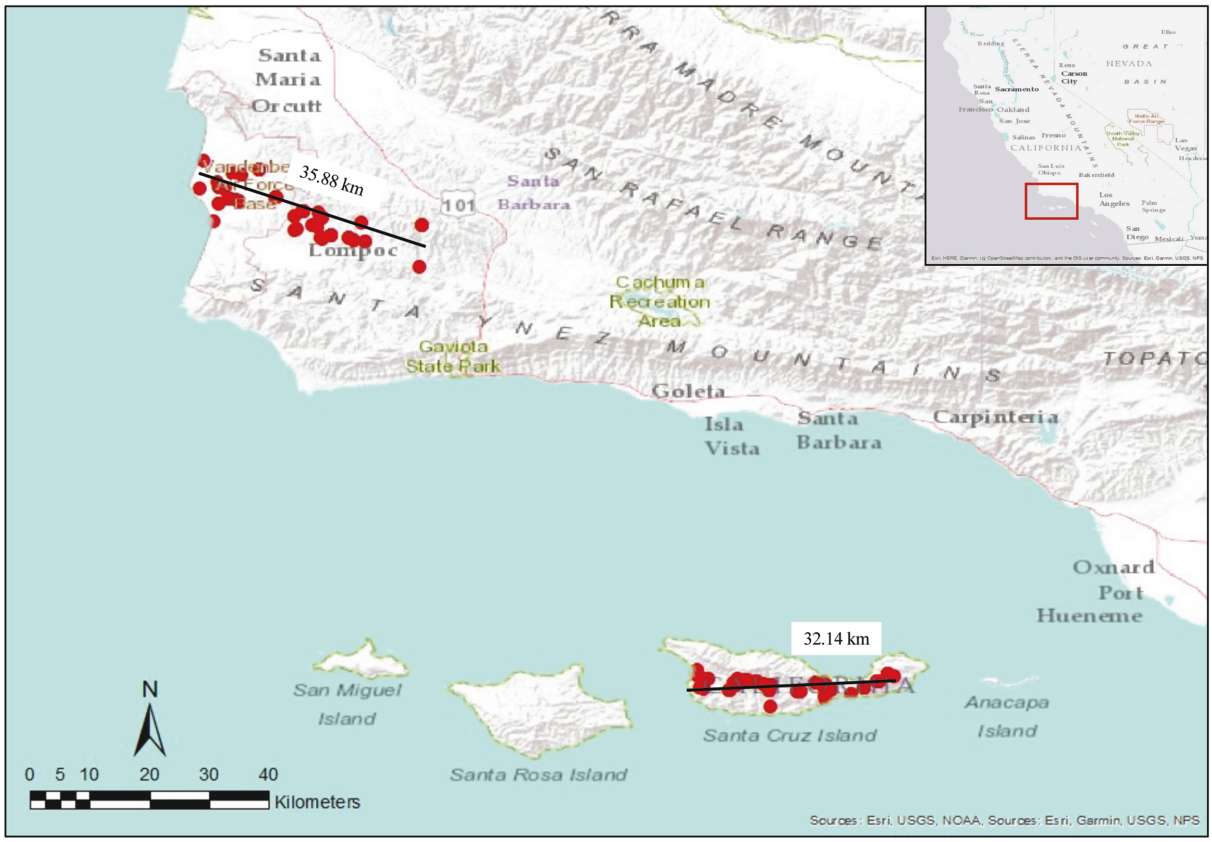

Figure 1. Map of sampling areas showing collection sites (red points) along linear transects. Some collection sites are not visible due to the layering of proximal points on top of each other; for GPS coordinates see Table S-1.

an assemblage of nine ant species, which broadly overlap in preferred habitat and often occur together (Naughton et al., 2020), yet they are phylogenetically dispersed across the Formicidae (Branstetter et al., 2017a) and are not congeneric. These nine species are mostly considered to be generalist foragers, competing for insect carrion, preying on arthropods, securing floral resources, and forming mutualisms with honeydew-producing aphids (Naughton, personal obs.). These species differ in their use of nesting sites. *Pheidole hyatti,* e.g., prefers to nest under stones, *Camponotus hyatti* inhabits buried dead wood, and *Dorymyrmex* us-ca05 nests in the soil in open ground. *Formica moki* and *Monomorium ergatogyna* are more variable, nesting under stones, directly in the soil, or in rotten wood. By comparing genetic diversity and population genetic structure between mainland and island populations across this assemblage of nine interacting ant species, our study clarifies the extent to which populations of species in an assemblage differ in response to evolutionary pressures in an island system and provides a test of whether general patterns of insularization are present.

Methods

Sampling and study area

We conducted sampling for this study on Santa Cruz Island, Santa Barbara Co., CA and a comparably sized area on the adjacent mainland (Lompoc Valley, Santa Barbara Co., CA). Santa Cruz Island (249 km^2 in area) lies 30 km from the mainland and has never been connected to the continent (Figure 1). As recently as the last glacial maximum, however, Santa Cruz Island and the remaining northern Channel Islands formed a single land mass that was separated from the mainland by approximately 7 km (Schoenherr et al., 2003). The Lompoc Valley, approximately 100 km northwest of Santa Cruz Island, encompasses the lower portions of the Santa Ynez River watershed. Island and mainland sampling areas resembled each other in terms of topography, climate, and vegetation types and also broadly overlapped in the species of native ants. We sampled each species from five to ten sites along an E–W transect (Figure 1; Table 1). Collected workers were placed directly into 95% EtOH and stored at –20°F, and vouchered specimens will be deposited in the Bohart Museum of Entomology at UC Davis. Collecting took place over multiple collecting trips to each area between March 2019 and August 2020; GPS coordinates for each sample are listed in Table S-1.

UCE library prep and bioinformatics

To generate genetic data for island-mainland comparisons, we conducted high-throughput sequencing of UCEs. We used Qiagen DNeasy Blood & Tissue kits (Valencia, CA) to extract total genomic DNA from one ant worker sample from each collection location (after removing gasters from all workers). Within the haplodiploid sex-determination system found in ants, all workers and queens in the colony are female and diploid, whereas males develop from unfertilized eggs and are haploid. We made the following modifications to the Qiagen kit protocol to optimize small amounts of starting tissue: samples were first ground on a bead mill for 1 min at 3200 rpm, then we added 50 µg RNase A and 10µL DTT to

the lysis step. We eluted samples in 300 µL RNase/DNase-free water, then concentrated samples to 100 µL using an Eppendorf Vacufuge. Following extraction, we quantified samples using an Invitrogen Qubit 1X dsDNA HS kit, then sheared samples using a Bioruptor sonicator (Diagenode) for one min total shearing time (15 s shearing time, 90 s rest for four repetitions). We used Sera-Mag Magnetic SpeedBeads in PEG mixture to clean sheared DNA samples to retrieve desired fragment sizes (400–900 bp in length). We used KAPA DNA Hyperprep kits to conduct end repair and A-tailing on each sample, then amplified each sample with Integrated DNA Technologies xGen UDI Primer Pairs and xGen Stubby Adapters, for 12 cycles using KAPA HiFi Hotstart Ready Mix. Following index PCR, we quantified libraries and visualized each library on a gel (1.5% agarose, 80 V for 60 min) to ensure target fragment sizes (400–900 bp) were obtained.

To perform targeted enrichment on pooled libraries, we used a UCE bait set of custom-designed probes (for ants) targeting 2,590 UCE loci (Branstetter et al., 2017a). We followed library enrichment procedures for the Arbor Biosciences MyBaits kit (Arbor Biosciences, Inc.) to set up bait hybridization, and then hybridized RNA baits to libraries at 65 °C for 24 hr. We amplified enriched libraries using universal Illumina primers and 18 PCR cycles, and purified PCR product using a 1.2X SPRI bead clean. To verify the enrichment of our libraries, we conducted a qPCR assay (Faircloth, 2013a) on five pairs per lane of sequencing of unenriched and postenriched libraries using DyNAmo Flash SYBRGreen qPCR kit (Thermo Fisher Scientific) to amplify three UCEs in each library (UCE82, UCE591, and UCE1481). After qPCR verification, we sent enriched samples to the Vincent J. Coates Genomic Sequencing lab at UC Berkeley where the peak fragment size of each pool was checked on a Bioanalyzer prior to pooling at equimolar concentrations into a single lane and then sequenced on an Illumina NovaSeq platform. Our samples were sequenced in two lanes of sequencing under the same protocols.

After sequence data were demultiplexed and converted to FASTQ format by the Vincent J. Coates Genomics Sequencing laboratory, we processed sequence data to obtain SNP data and UCE alignments for sample sets of each species; each species sample set included sequence data of one individual worker per collection site along each of the mainland and island transects (Figure 1). We used ILLUMIPROCESSOR (Faircloth, 2013b) to clean and trim raw FASTQ reads and to remove low quality reads. To maximize the number of UCE regions recovered and the length of the flanking regions, we used SPAdes (Prjibelski et al., 2020) to assemble contigs with a range of *k*-mers of 21, 33, and 55, and then selected the longest contig for overlapping UCEs. Statistics on assembly size and coverage were calculated using the Phyluce mapping workflow (Faircloth, 2016) and are included in Table S-2. We matched assembled contigs to UCE loci and generated a sqlite database of all UCE reads for each sample using the PHYLUCE program phyluce_align_match_contigs_to_probes, then aligned all loci in a wrapper script (phyluce_align_seqcap_align) around MAFFT v.7.130b (Katoh & Standley, 2013). We retained loci that contained 75% or more of our samples for allele phasing.

To obtain data for estimates of heterozygosity, genetic distance, and F_{st}, and to construct STRUCTURE plots, we phased our UCE sequences and extracted one SNP per UCE locus from phased reads for downstream analyses. Allele phasing effectively identifies variable positions within a target locus of an individual; these positions are typically lost during contig assembly, as most assembly algorithms produce only the more numerous variants while discarding alternative variants (Andermann et al., 2019). For each sample, we mapped raw fastq reads against reference contigs and marked read duplicates with SAMtools (Li, 2011), added read groups with Picard (http://broadinstitute.github.io/picard/), and constructed a BAM file using bwa-mem (Li & Durbin, 2009). We used the Phyluce script phyluce_snp_phase_uces to analyze and sort reads within the BAM file for each sample into reads for each allele and create fasta files for UCE reads. We aligned phased fasta files, then called SNPs using the Phyluce script phyluce_snp_screen_phased_alignments. We then used a custom Python script (https://github.com/dportik/Convert-fasta-alignments-to-Structure-format) to extract one SNP per locus at random with the -remove_singletons flag which prevents the selection of single SNPs from alignments, in order to eliminate SNPs that may have originated via sequencing errors and the potential for singleton SNPs to confound detection of population subdivision (Linck & Battey, 2019), and also without the -remove_singletons flag, and ran subsequent summary statistics on both datasets. For the SNP dataset of each species, we plotted the site frequency spectra (SFS) to observe the frequencies of SNPs captured in our final data matrices. Interestingly, we observed that both *M. ergatogyna* and *Tapinoma sessile* contained numerous SNPs with a frequency of 0.5 (557 and 419 sites, respectively), and when we observed these sites within the aligned, phased UCE loci from which they were extracted, we found each individual (mainland and island populations) to be heterozygous at the SNP site. Given that we were unable to determine the origin of these fixed heterozygotes, we filtered out the UCE loci containing fixed heterozygotes for both of the *T. sessile* and *M. ergatogyna* datasets. Results of the analyses with the UCE loci containing fixed heterozygotes are included in Table S-3.

In addition to matrices of SNP data, we constructed alignments of UCE loci for each species to examine the degree of genetic polymorphism within populations. We used the sqlite database generated from matching assembled contigs to UCE probes to generate separate monolithic fasta files for all samples of each species, retained loci that contained 75% or more samples, and aligned all loci using a wrapper script (phyluce_align_seqcap_align) around MAFFT v.7.130b (Katoh & Standley, 2013); retained UCE data matrices ranged from 1,660 to 2,386 (Table 1). For *M. ergatogyna* and *T. sessile,* we filtered loci that contained one or more SNP with a fixed heterozygous site and ran downstream analyses on both filtered and unfiltered alignments. We ran summary statistics on the full dataset with and without the *M. ergatogyna* and *T. sessile* fixed heterozygous SNPs included; the results did not differ significantly (Table S-3).

Genetic diversity analyses

We selected several measures to use in tests of differences in genetic diversity between mainland and island populations. To obtain information about the levels of genetic variation in each population, we obtained measures of observed and expected heterozygosity for mainland and island populations of each species. All measures of heterozygosity were calculated based on SNP datasets using Adegenet 2.1.3 (Jombart, 2008) implemented in R 3.6.2 (R Core Team, 2019), with and without singletons included. To determine the degree of genetic

Table 2. Genetic diversity and population genetic structure measurements for island and mainland populations of nine ant species. Ho, He, and Nei's D estimates are based on SNP data (one SNP per UCE locus). Watterson's theta estimates are based on aligned UCE reads for each species, and raw Watterson's theta estimates are log-transformed. A Wilcoxon signed rank exact test was used to compare He–Ho values between mainland and island populations; paired *t*-tests were used to conduct assemblage-level comparisons for all other measurements.

Species	Ho		He		He–Ho		Nei's D		Watterson's theta	
	Island	Mainland	Island	Mainland	Island	Mainland	Island	Mainland	log (island)	log (mainland)
Monomorium ergatogyna	0.2198	0.2051	0.2711	0.3061	0.0513	0.1010	0.1272	0.1851	0.2219	0.3049
Prenolepis imparis	0.1757	0.1776	0.2401	0.2368	0.0644	0.0592	0.0514	0.0612	–0.0071	0.0420
Solenopsis molesta	0.1304	0.1211	0.2606	0.3832	0.2528	0.2621	0.1392	0.2568	0.0363	0.2333
Crematogaster marioni	0.1432	0.1861	0.2736	0.2817	0.1385	0.0956	0.1547	0.1127	0.1671	0.0587
Tapinoma sessile	0.1806	0.1359	0.1780	0.2169	–0.0026	0.0810	0.0362	0.1271	–0.1890	0.1194
Pheidole hyatti	0.1761	0.1630	0.2577	0.2895	0.1134	0.1265	0.0863	0.1328	0.1428	0.3510
Formica moki	0.1311	0.1546	0.1828	0.2233	0.0922	0.0687	0.0499	0.0884	0.1369	0.6236
Dorymyrmex us-ca05	0.0669	0.0518	0.1605	0.1601	0.0932	0.1083	0.0938	0.1105	–0.2063	0.0371
Camponotus hyatti	0.0793	0.1366	0.0998	0.2832	0.2039	0.1466	0.0168	0.1194	–0.1766	0.3064
Mean	0.1448	0.1480	0.2138	0.2645	0.1119	0.1166	0.0839	0.1327	0.0140	0.2307
SE	0.0165	0.0145	0.0203	0.0213	0.0260	0.0203	0.0162	0.0910	0.0556	0.0640
T	0.2972		2.4463		.8203		2.895		3.3289	
***p*-value**	.7739		**.0402**				**.0200**		**.0104**	

Note. Purple columns show measures of island populations, orange columns show measures of mainland populations.
p-values for *t*-tests less than .05 are shown in bold.

polymorphism in populations, we calculated Watterson's theta on alignments of UCE loci using the R package PopGenome 2.7.5 (Pfeifer et al., 2014) and log-transformed the raw values in order to fit a normal distribution. To compare values of genetic diversity and differentiation between mainland and island populations, we used a Shapiro–Wilks test (Table S-4) to check that differences between paired populations (island–mainland) were normally distributed and then performed paired *t*-tests to test for assemblage-level differences between island and mainland populations. For the comparison of differences between expected and observed heterozygosity between mainland and island populations (He—Ho), we used a Wilcoxon signed rank exact test.

Population structure analyses

To measure genetic structuring within populations, we calculated average pairwise genetic distance (Nei's standard genetic distance (Nei, 1987)) using Adegenet 2.1.3 (Jombart, 2008) implemented in R 3.6.2 (R Core Team, 2019). To examine levels of gene flow and structuring between island and mainland populations, we calculated F_{st} between island and mainland populations for each species using Adegenet 2.1.3 (Jombart, 2008). To visualize levels of genetic structuring between mainland and island populations, and within each population, we analyzed SNP data under the admixture model in STRUCTURE 2.3.4 (Pritchard et al., 2000), and parallelized the STRUCTURE runs using StrAuto 1.0 (Chhatre & Emerson, 2017). We assumed different numbers of genetic demes from $K = 1$ to $K = 6$ for 100,000 generations with a burn-in of 50,000 and three replicates at each value of K. We used STRUCTURE-Harvester (Earl & VonHoldt, 2012) to determine the most likely number of genetic demes based on the Evanno method (Evanno et al., 2005) (Table S-5). We used the *dudi.pca* function in ade4 1.7-22 (Dray & Dufour, 2007) to perform principal component analyses (PCAs) on the SNP dataset for each species, and plotted the results in R 3.6.2 (R Core Team, 2019).

Given that molecular phylogenetics is also a useful tool for examining gene flow in island systems (Emerson, 2002), we used alignments of UCE sequences to assess the monophyly of island and mainland populations. As outgroups to root the phylogenies, we chose UCE sequences generated from the same UCE probe set (Branstetter et al., 2017a) from the NCBI Sequence Read Archive (SRA) of geographically distant conspecific samples (Borowiec et al., 2021; Branstetter et al., 2017a, 2017b; Oberski, 2022, 2023; Tonione et al., 2022; Ward & Blaimer, 2022) or from UCE sequence data held by coauthors (Table S-6). We filtered our unphased UCEs plus the additional outgroup for each dataset to loci contained in 95% or more samples and aligned all loci using a wrapper script (phyluce_align_seqcap_align) around MAFFT v.7.130b (Katoh & Standley, 2013). We used IQ-tree for phylogenetic analyses for each species dataset, using a bootstrap search of 1,000 ultrafast bootstrap replicates (Hoang et al., 2018) and an edge-unlinked partitioned model with each UCE sequence as a separate partition. In addition to our key analyses, we provide additional measures of genetic diversity and differentiation in Table S-7.

Gyne morphology and population structure

To determine whether gyne morphology can predict population structure and gene flow between mainland and island populations, we tested for relationships between two

measures of population structuring (F_{st} between island and mainland populations and Nei's D within mainland populations) and two morphological measures (Weber's length and wing length) that presumably influence dispersal ability. Weber's length (taken from the anterodorsal margin of the pronotum to the posteroventral margin of the propodeum) is a commonly used proxy for body size (Brown, 1953; Gotelli & Ellison, 2002; Helms, 2018), whereas wing length influences dispersal ability in insects in general (Greenleaf et al., 2007; Harrison, 1980). To obtain morphological measurements for winged gynes, we measured two museum specimens from mainland populations for each species except for *M. ergatogyna*; winged gynes appear rare in this species, and we were only able to obtain a wing measurement from one individual. Since we were only able to acquire two winged gynes for each species (and only one for *M. ergatogyna*) from the mainland, our sampling design overlooks intraspecific variation of gynes across species and differences between mainland and island populations, thus the results of our analyses represent preliminary findings on the relationship between gyne body size and dispersal ability. However, given that measurements of gyne body size vary greatly across the species here (Table S-8), we expect that the magnitude of interspecific variation would substantially outweigh the effects of intraspecific variation and that the relationships described here would be upheld in more rigorous analysis. We used linear regressions to test for relationships between gyne morphology (Weber's length and wing length) and genetic structure (F_{st} between mainland and island populations and Nei's D within populations).

Results

Across the nine species in our study, mainland and island populations differed from one another in terms of population genetic structure and genetic diversity. Our first hypothesis was supported in that measures of genetic diversity, such as expected heterozygosity (He), were significantly lower in island populations compared to conspecific mainland populations, although observed heterozygosity (Ho) did not differ (Table 2, Figure 2). Expected heterozygosity exceeded observed heterozygosity in both mainland and island populations for each species except for the island population of *T. sessile* (Table 2). Neither observed nor expected heterozygosity significantly differed when singleton SNPs were included in the analysis of summary statistics (Table S-9), although most species exhibited higher He and Ho in mainland populations than island populations. Additional evidence that mainland populations supported greater genetic diversity came from measures of Watterson's theta, which is based on polymorphisms between aligned UCE loci.

In addition to higher levels of genetic diversity, mainland populations had higher values of pairwise genetic distance compared to those of island populations (Table 2, Figure 2). Contrary to our second hypothesis that island populations would exhibit higher population genetic structure, measures of average pairwise genetic distance (Nei's D) ranged from 0.0362 (*T. sessile*) to 0.1547 (*Crematogaster marioni*) in island populations, and from 0.0612 (*Prenolepis imparis*) to 0.2568 (*Solenopsis molesta*) in mainland populations (Table 2). Estimates of average pairwise genetic distance were higher within mainland populations across all species except for *C. marioni*, which showed the opposite pattern.

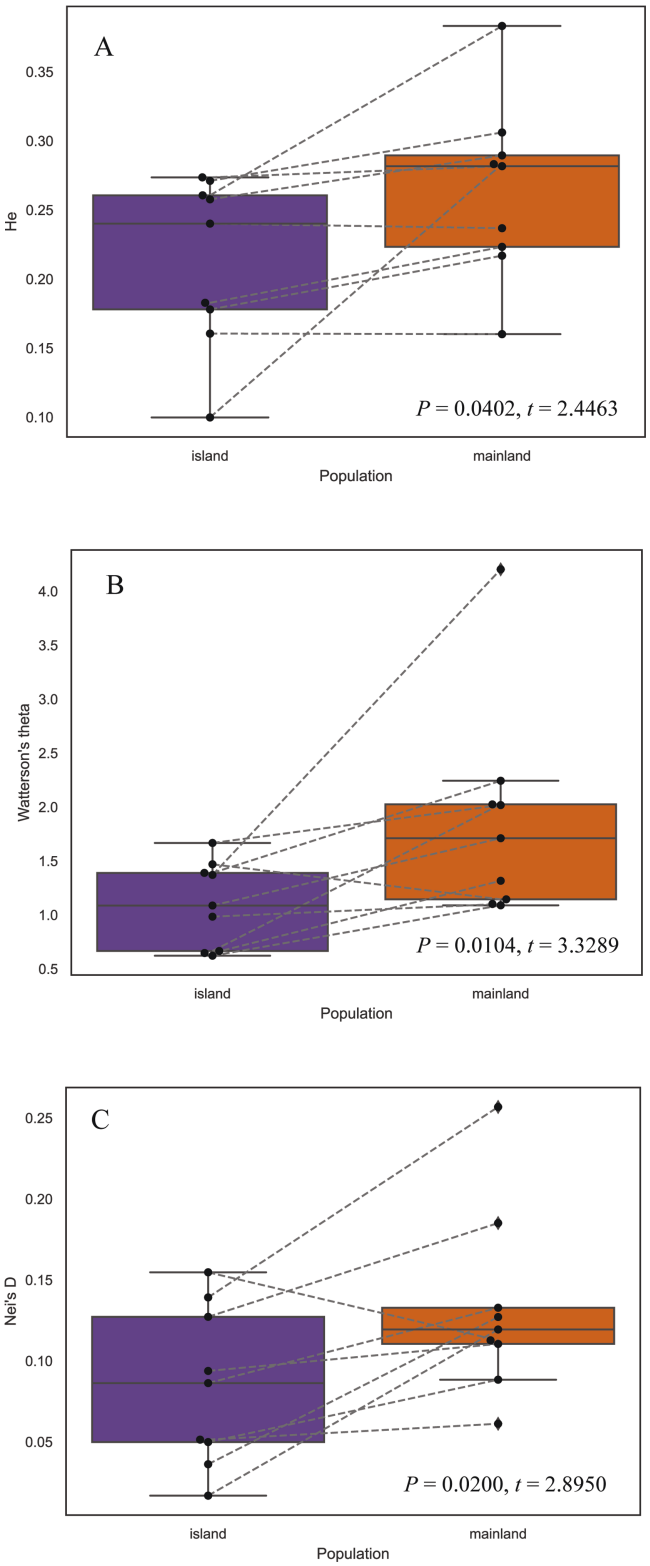

Figure 2. Boxplots summarizing differences between island (Santa Cruz Island) and mainland (Lompoc Valley) populations with respect to (A) expected heterozygosity (B) Watterson's theta (C) within-population Nei's D. Lines connect island-mainland pairs of each species. Paired *t*-tests from Table 2.

Analyses of genetic admixture between mainland and island populations for each species revealed striking interspecific variation with respect to the degree of genetic differentiation.

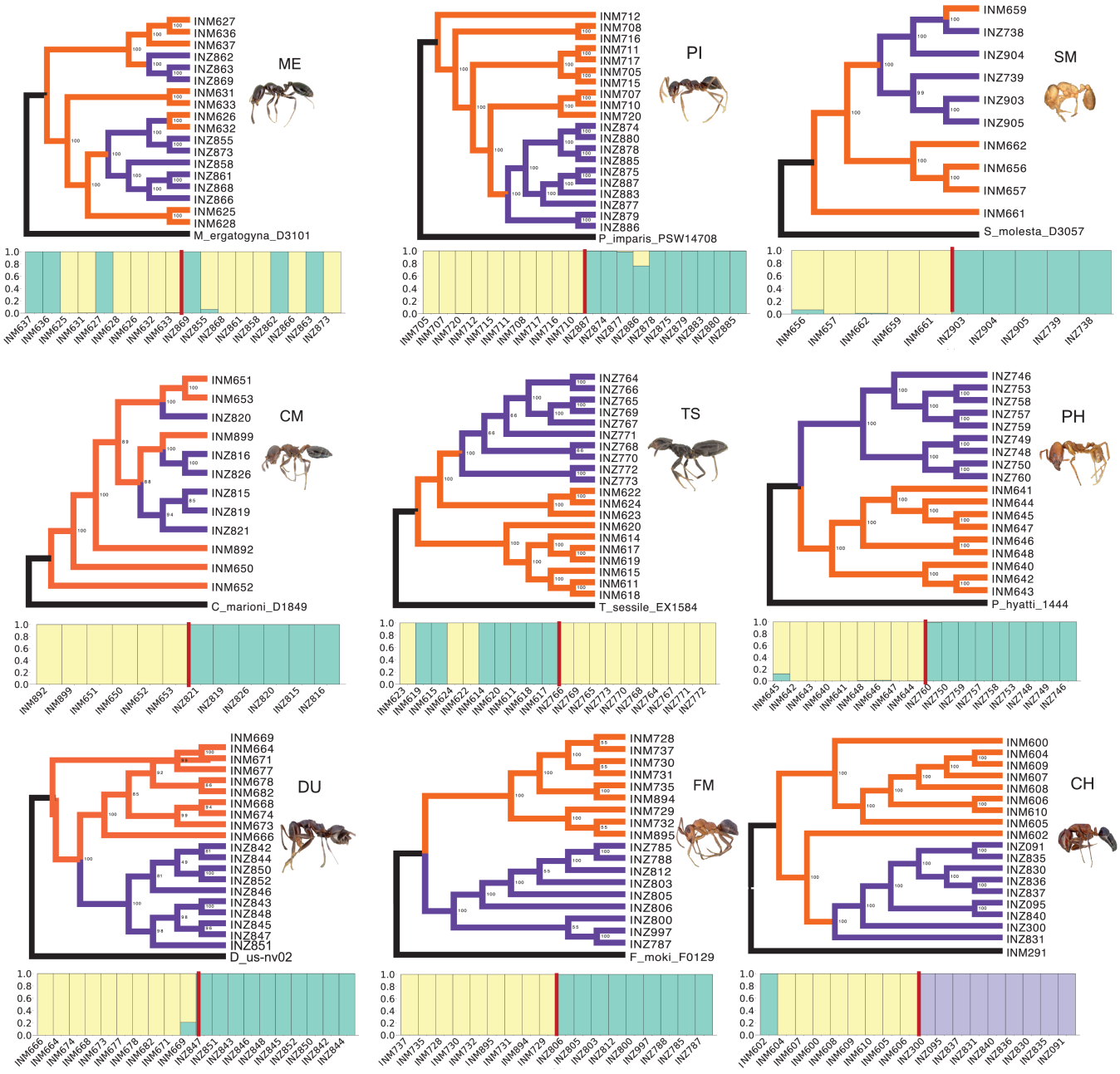

Figure 3. IQ-Trees (cladograms) based on aligned UCEs (top), and STRUCTURE plots based on SNP data (bottom) at the most likely number of populations (*K*) based on the Evanno method (Evanno et al., 2005) for each mainland (Lompoc Valley) and island (Santa Cruz Island) population of each species. See Table 1 for species codes and Figure S-2 for trees with branch lengths. For STRUCTURE plots, samples are arranged west-to-east within each transect (island or mainland), and bold lines separate mainland (left of red line) and island (right of red line) samples. STRUCTURE plots show the most likely value of K based on the Evanno method ($K = 2$ for all species except CH, in which $K = 3$). The STRUCTURE plot of CH at $K = 2$ is available in Figure S-3. Images were sourced from AntWeb, n.d. Version 8.95.1.: www.antweb.org. Photo credits: ME—Michael Branstetter, PI—April Nobile, SM—Zachary Griebenow, CM—Xiaofan Yang, TS—Shannon Hartman, PH—April Nobile, FM—April Nobile, DI—April Nobile, CH—April Nobile.

Mainland and island samples of *C. hyatti* and *D.* us-ca05, e.g., were separated into distinct genetic demes at the most likely value of *K* within STRUCTURE plots, exhibited monophyletic groupings in the IQ-trees (Figure 3, Figure S-3), and formed distinct clusters within the PCAs (Figure 4). *C. hyatti* and *D.* us-ca05 also exhibited high pairwise F_{st} values (0.4614 and 0.3183, respectively) between mainland and island populations. In contrast, *M. ergatogyna* exhibited admixture between island and mainland populations within STRUCTURE plots and IQ-Trees (Figure 3, Figure S-2), and a relatively low pairwise F_{st} value between mainland and island populations (–0.0024). Additionally, the phylogenetic analysis of *M. ergatogyna* revealed a paraphyletic grouping of mainland samples with respect to island samples (Figure 3, Figure S-2). Within STRUCTURE plots, samples for most species formed distinct genetic demes representing island and mainland samples at $K = 2$ (Figure 3, Figure S-3), suggesting that mainland and island populations are distinctive even for species that exhibit relatively high levels of mainland-island gene flow.

We also found support for our third hypothesis that mainland–island differentiation increases with increasing gyne size, and that within-mainland differentiation decreases with gyne

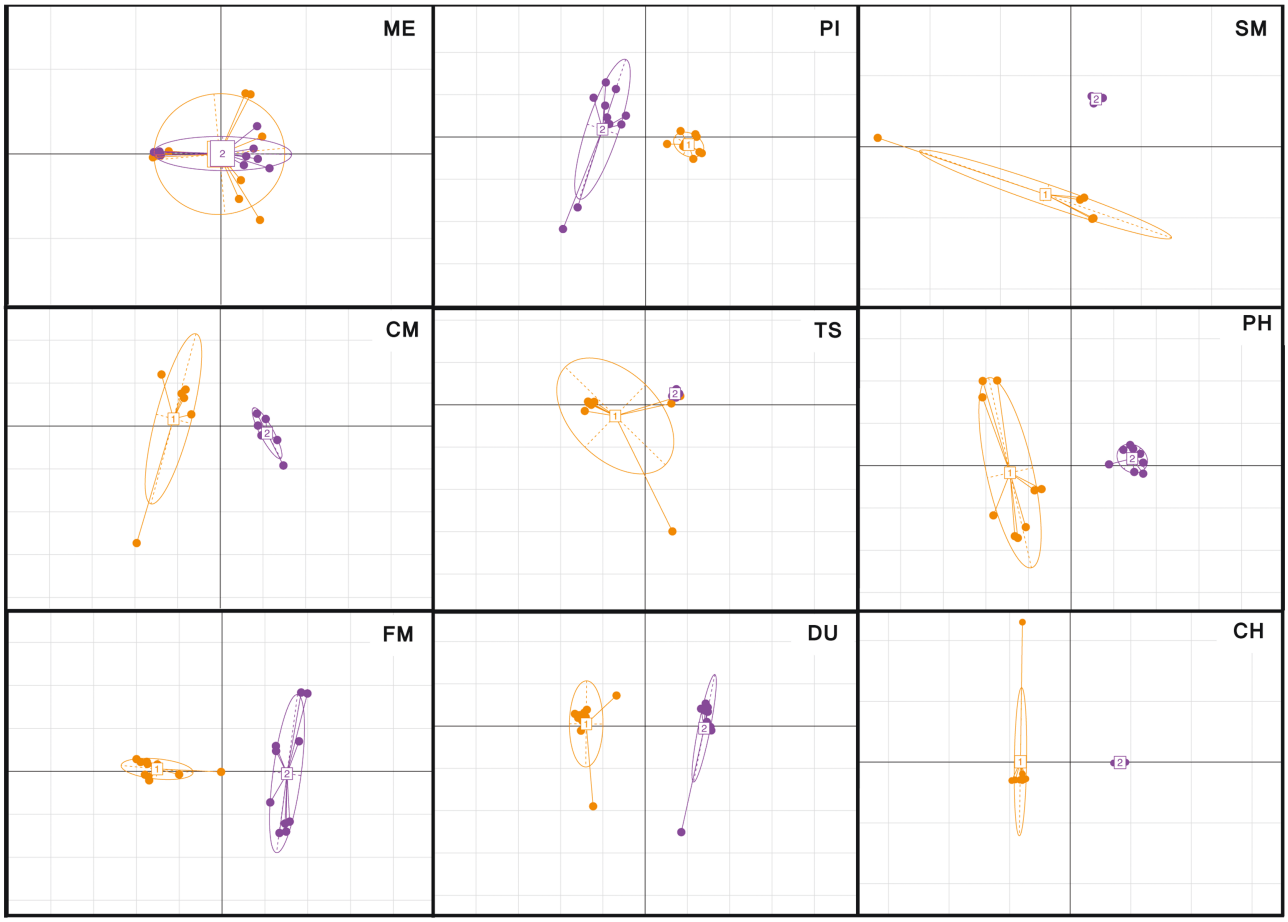

Figure 4. Principal component analyses (PCAs) based on SNP data for each species. Orange points, and clusters marked as "1" represent mainland samples; purple points and clusters marked as "2" represent island samples. See Table 1 for species codes.

size. Measures of F_{st} between mainland and island populations increased with Weber's length ($F_{1,7} = 7.377, p = .029$, Figure 5) and wing length ($F_{1,7} = 6.223, p = .041$). Additionally, Nei's D decreased with Weber's length ($F_{1,7} = 6.639, p = .036$, Figure 5) and possibly also with wing length ($F_{1,7} = 5.055, p = .059$) within mainland populations.

Discussion

Our assemblage-level comparison of genetic diversity and population structure between island and mainland populations of nine ant species provides unexpected insights into how evolutionary forces act within a set of interacting species. Consistent with our first hypothesis, genetic diversity was higher at the level of the assemblage in mainland populations, compared to island populations, across all diversity metrics except observed heterozygosity (Ho). This finding largely conforms to theoretical expectations; island populations typically support less genetic diversity compared to mainland populations as a result of smaller population sizes, founder effects, population bottlenecks, and reduced immigration. Contrary to our second hypothesis, we found that mainland populations supported greater population genetic structuring at the level of the assemblage compared to island populations. This finding suggests a higher capacity for local dispersal within island populations compared to that of mainland populations. Lastly, gyne body size appeared to affect dispersal: F_{st} between mainland and island populations increased with gyne size yet intra-population genetic distances decreased with gyne size within mainland populations.

Differences in genetic diversity

Mainland populations often support higher levels of genetic diversity compared to island populations (Frankham, 1997; Whittaker & Fernández-Palacios, 2007), likely as a result of founder effects and population bottlenecks. Our results are consistent with this expectation; ant populations on islands exhibited reduced levels of genetic diversity in terms of expected heterozygosity and Watterson's theta compared to those on the mainland. According to island biogeography theory, mainland populations are larger and older than island populations, and complex demographic histories could account for higher levels of heterozygosity compared to those observed in island populations (Hahn, 2019). Higher estimates of Watterson's theta in mainland populations, e.g., suggest that these populations are larger and thus less likely to be affected by genetic drift compared to island populations. Interestingly, although expected heterozygosity was significantly higher in mainland populations, observed heterozygosity did not significantly differ between mainland and island populations. Greater population genetic structure within mainland populations (as evidenced by higher estimates of Nei's D) can increase differences in allele frequencies between subpopulations, and in turn elevate levels of

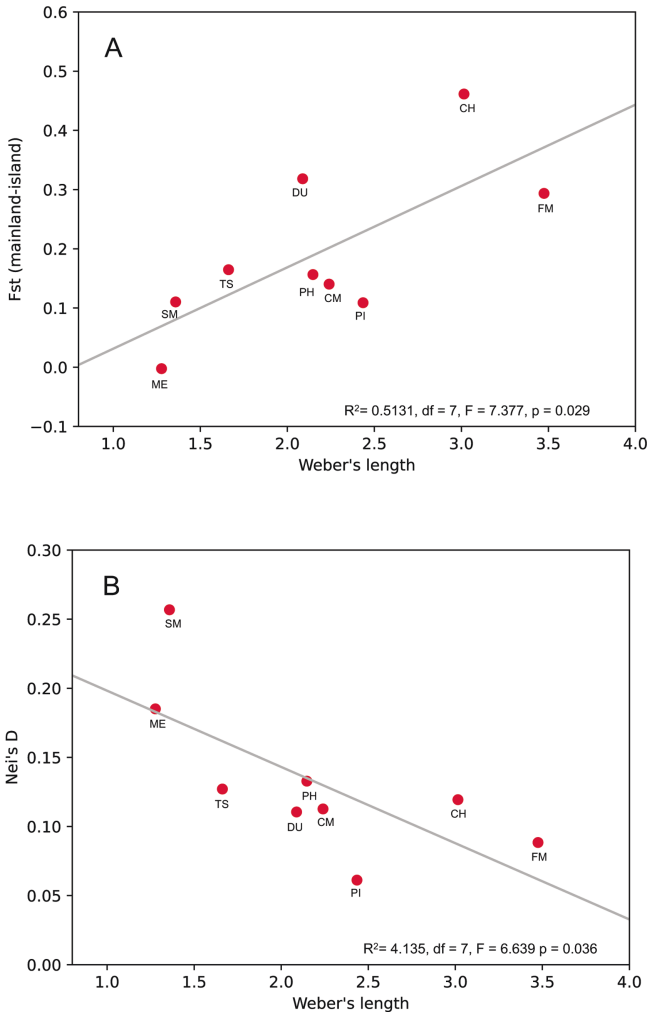

Figure 5. (A) F_{st} between mainland and island populations vs. Weber's length (simple linear regression: $F1,7 = 7.377$, $p = .029$, $R^2 = 0.513$). (B) mainland population measures of Nei's D vs. Weber's length (simple linear regression: $F1,7 = 6.639$, $p = .036$, $R^2 = 4.135$). See Table 1 for species codes.

expected heterozygosity (Meirmans & Hedrick, 2011). Our results provide further evidence that colonization of offshore islands may give rise to populations with less genetic diversity compared to conspecific mainland populations. This finding is crucial to the conservation management of island ecosystems, given that insular populations may be especially prone to extirpation given their population size and exposure to environmental change (Gillespie & Roderick, 2002; Rick et al., 2014).

The genetic diversity of island populations can be strongly influenced by species interactions (Gillespie, 2004). Establishment on islands can result in ecological release via the absence of competitors (Cole, 1983), and expansion of the realized niche can change selection pressures and affect genetic diversity. A potential example from our system concerns *C. marioni*, which was an outlier in comparisons of genetic diversity and population genetic structure, in that it was the only species for which measures Watterson's theta were higher within island populations compared to mainland populations. On the mainland, *C. marioni* is uncommon and nests arboreally, often in coast live oak (*Quercus agrifolia*). On Santa Cruz Island, this species is common, occurs in a variety of habitats, and exhibits a wider variety of nesting habits, including ground nesting. These contrasting patterns plausibly result from competitive release from mainland competitors, such as the velvety tree ant (*Liometopum occidentale*), which is abundant in oak woodland on the mainland but absent from the California Channel Islands.

Genetic structuring within and between populations

Although the reduction in dispersal ability is widely considered to be a feature of the island syndrome, further studies are required to determine whether or not dispersal ability is typically lower on islands (Waters et al., 2020). Here we show evidence for an island assemblage with lower levels of genetic structuring compared to the mainland assemblage; this finding is consistent with a decreased capacity for dispersal. Measures of genetic distance (Nei's D) were significantly lower in island populations of every species examined except for *C. marioni*. This finding seems surprising given that organisms that rely on passive long-distance aerial dispersal can undergo a reduction in dispersal ability after they colonize islands (Cody & Overton, 1996; Wauters et al., 2018). Although differences in wind intensity between island and mainland sites could contribute to observed differences in dispersal ability (Pasek, 1988), especially if these differences overlap with the timing of nuptial flights, wind patterns between Santa Cruz Island and Lompoc do not appear to differ (Figure S-1). In addition to dispersal capacity, population age and size can influence measures of genetic structure such as Nei's D (Hahn, 2019). In the context of taxon cycle dynamics, e.g., heightened dispersal abilities within island populations could indicate relatively recent colonization, in which island populations enter a phase of expansion and high population connectivity initially, but subsequently undergo relictualization and loss of vagility within the island over time (Economo & Sarnat, 2012; Wilson, 1961).

The degree of gene flow from continental populations profoundly affects the genetic diversity of island populations (Losos & Ricklefs, 2009; MacArthur & Wilson, 1967). In this study, measures of F_{st} and STRUCTURE plots revealed that the degree of differentiation between mainland and island populations differs widely among species, reflecting strong interspecific differences in the degree of mainland-island gene flow. *Tapinoma sessile* and *M. ergatogyna* were the only two species for which mainland and island populations did not form distinct genetic demes at $K = 2$ (Figure 3, Figure S-2). The origin of these divergent genetic groups seems unclear. However, both *T. sessile* and *M. ergatogyna* had numerous fixed heterozygous sites across UCE loci, which were filtered out of the analysis; these fixed sites may be a consequence of parthenogenetic or thelytokous reproduction (Rabeling & Kronauer, 2013). Such unusual reproductive modes are known from other ant species (Idogawa et al., 2021; Rabeling & Kronauer, 2013), and could give rise to fixed heterozygous sites that would, in turn, produce unexpected signatures of population connectivity. Although these sites could also represent paralogs or artifacts of bioinformatic processing, this seems unlikely given that we only found fixed sites in these two species and used the same bioinformatic processing methods for all nine species. Further study is necessary to determine the reproductive modes of *T. sessile* and *M. ergatogyna* and to clarify the origins of fixed heterozygous sites across the genomes of these two species.

In addition to population genetic analyses of SNP data, phylogenetics can clarify whether or not island populations are the result of single or multiple colonization events (Emerson, 2002). Island populations in this study appear to have originated via multiple colonization events between the mainland and island in three species (*M. ergatogyna*, *S. molesta*, and *C. marioni*) based on IQ-Tree analyses, but for the remainder of this assemblage, phylogenies are consistent with single colonization events. Interestingly, for *C. marioni* and *S. molesta*, island and mainland populations formed distinct genetic demes in the STRUCTURE plots, in contrast to paraphyletic groupings of island and mainland populations in the IQ-Trees. This finding suggests that the SNP datasets for these species, which include one SNP per locus, may overlook informative sites that are captured in the IQ-Trees, which analyze polymorphisms across each UCE locus. Moreover, STRUCTURE and IQ-Tree use very different assumptions and models (i.e., admixture vs. substitution models, respectively) to provide different, complementary insights into the focal populations. Thus, the STRUCTURE results may reflect patterns of more recent gene flow or drift whereas the phylogenetic reconstruction can capture deeper phylogenetic history. Additionally, the discrepancies between IQ-Trees and STRUCTURE plots are less pronounced at higher values of K for *S. molesta* and *C. marioni* (Figure S-3). Together, phylogenetic analyses of island and mainland populations presented here help to clarify interspecific differences in mainland-island differentiation across our focal assemblage.

Interspecific differences in dispersal ability

At low altitudes, the capacity for powered flight increases with body size for insects generally (Dillon et al., 2006; Dudley, 2002), and ants in particular (Helms & Kaspari, 2014, 2015). Consistent with this hypothesis, our results show a negative relationship between population differentiation (average pairwise Nei's D) and body size within mainland populations, suggesting that species with larger gynes exhibit a higher capacity for local dispersal (which would increase gene flow) within populations. Although population size also affects estimates of F_{st}, F_{st} between mainland and island populations increased with gyne body size (Weber's length) suggesting that the Santa Barbara Channel may be a more formidable dispersal barrier for larger species. Additionally, wind-assisted dispersal could potentially increase dispersal distance for small gynes in particular, given that they are more likely to fly at higher altitudes (Dillon et al., 2006; Dudley, 2002; Helms et al., 2016). Aerial dispersal of winged reproductives seems more important than rafting as a means of colonization given the presence of winged alates in all but one species in the study, prevailing northwesterly winds in the Santa Barbara Channel (Schoenherr et al., 2003), and the strictly ground nesting habit of some species in the study. Interestingly, the only species in the assemblage that typically produces ergatoid (wingless) queens is *M. ergatogyna*, which exhibits the lowest F_{st} between mainland and island populations, suggesting a lack of genetic structuring between mainland and island populations. *Monomorium ergatogyna* is also the only ant species to occur on all eight of the California Channel Islands (and some adjacent islets), suggesting an exceptional ability to achieve overwater dispersal and establishment on islands. Although we were unable to acquire a set of male alates for all of the species in this study, male dispersal is also likely important to gene flow, thus a comprehensive analysis of dispersal of both males and females would further clarify routes of gene flow in this system. Ants exhibit striking interspecific variation with respect to alate morphology, dispersal behavior, and colony founding syndromes (Enzmann & Nonacs, 2010; Helms & Kaspari, 2014, 2015; Hölldobler & Wilson, 1990; Shik & Kaspari, 2009), and studies that link morphological and behavioral characteristics of winged reproductives with population genomic data will help elucidate the traits that correspond with differing capacities for dispersal and colonization of offshore islands.

Studies on ants have long provided insights into community assembly dynamics in insular systems (Cole, 1983; MacArthur & Wilson, 1967; Vepsäläinen & Pisarski, 1982; Wilson, 1961). This study contributes to this body of work by highlighting how patterns of dispersal from mainland populations influence community assembly in an island system. Populations with high genetic diversity may be more effective at establishing variable habitats throughout an island system (Mijangos et al., 2015). For example, different intraspecific genotypes may confer fitness advantages in competition with other species (Tsutsui et al., 2003; Vellend, 2006) or promote ecological function in variable environments (Reynolds et al., 2012). Additionally, a higher capacity for dispersal may promote intrapopulation connectivity and decrease the likelihood of local extinctions (Alors et al., 2017), but passive long-distance dispersal within island populations can lead to propagules being transported away from suitable habitats (Carlquist, 1980; Roff, 1986). Our results did not provide evidence for the selection of reduced capacity for dispersal in this island system, as island populations exhibited more population connectivity compared to mainland populations. Further study of species-level patterns of dispersal that include timing and frequency of nuptial flights, colony founding strategy, and male dispersal morphology will increase an understanding of the ways in which patterns of dispersal can contribute to genetic diversity and community assembly on islands.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Sequence data for all samples are available from the NCBI Sequence Read Archive (SRA) under BioProject ID: PRNJNA1032137. The following link has been generated for reviewer access to the BioProject, BioSample, and associated metadata: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1032137?reviewer=1bhjf7pstbkn46j58vjcig2fsv

Morphometric measurements for all museum specimens and all supplementary files are available on Dryad: https://doi.org/10.5061/dryad.2v6wwpzw7

Author contributions

I.N. and D.A.H. designed the study. I.N. conducted field sampling and molecular work. P.S.W. contributed sequence data and conducted morphometric measurements. I.N. analyzed the data with contributions from N.D.T, P.S.W, and D.A.H. I.N. and D.A.H. wrote the manuscript with contributions from N.D.T and P.S.W.

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