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GENETIC ANALYSIS OF DISPERSAL DYNAMICS IN AN INVADING POPULATION OF ARGENTINE ANTS

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Abstract. The ecology and dispersal dynamics of unicolonial ant invaders are poorly understood. In this study, we investigated the genetic structure of a well-documented, invading population of Argentine ants in the Jasper Ridge Biological Preserve in northern California to examine the dispersal distances of reproductives, the direction and mode of population expansion, and changes in the genetic differentiation among nests over time. Using microsatellite data, we measure both traditional F_{ST} statistics and multi-locus genotype assignment distances to determine the patterns of genetic structure at three spatial scales: population-wide gene flow, population substructure, and mixing between neighboring nests. At the population level, there was little viscosity across the Jasper Ridge population, suggesting recent rapid expansion and/or considerable long-distance gene flow, presumably mediated by winged males. The pattern of genetic structure across distance indicates that the scale of queen dispersal was limited to less than 100 m. At the level of population substructure, hierarchical *F* statistics measures were low across subpopulations, locations within subpopulations, and nests. However, multi-locus genotype assignment tests revealed significant structure between subpopulations and between locations. Genetic distances between nests were lower within locations than between locations, indicating that nests are most closely related to neighboring nests and that the expansion of subpopulations is primarily due to the local budding of new nests from existing nests at the invasion front. At the level of nest connectivity, the genetic differentiation among neighboring nests was associated with the time since invasion. As the invasion proceeds, nearby nests tend to be less closely related, indicating that extensive local mixing does not occur among more established nests. Our results show that the genetic structure of nests was not homogenous across unicolonial populations. Instead, the patterns of genetic structure reflect the limitations of and barriers to the dispersal of Argentine ant reproductives, the demographic history of the Jasper Ridge invasion, and changes in the genetic and ecological environment during the course of the invasion.

Key words: Argentine ants; dispersal; genetic structure; invasions; Linepithema humile*; microsatellites; social structure; supercolony; unicoloniality.*

INTRODUCTION

Invasions of unicolonial ants disrupt local arthropod communities (Ward 1987, Cole et al. 1992, Human and Gordon 1997, Holway 1998, Suarez et al. 1998), cause considerable economic damage to agricultural crops (DeBach and Rosen 1991), and create a major inconvenience inside homes (Gordon et al. 2001). Despite the numerous adverse effects of invasive ants, little is known about the social structure and ecology of unicolonial species in native or introduced populations. In general, unicolonial ant nests are characterized by the presence of multiple queens, low relatedness of nestmates, and low levels of aggression between neighboring nests (Passera 1994, Keller 1995). In introduced populations, the lack of clear colony boundaries and considerable mixing of individuals among nests can lead to the formation of supercolonies of many nests that may extend over thousands of kilometers (Tsutsui et al. 2000, 2001, Giraud et al. 2002). The fluid colony organization of unicolonial ants may contribute to their overwhelming ecological success.

One unicolonial invader, the Argentine ant, *Linepithema humile*, has become a notorious pest of agricultural, urban, and natural areas where it has been introduced around the world (Newell and Barber 1913, Markin 1970, Ward 1987, Cole et al. 1992, Human and Gordon 1996, Sanders et al. 2001). Many aspects of the population ecology of this species are not well understood, such as the dispersal distances of reproductives, the direction and mode of population expansion, and whether dispersal patterns change during the course of an invasion. Like many unicolonial ant species, Argentine ant queens shed their wings soon after eclosion and thus cannot disperse by flight (Markin 1970). Male reproductives retain their wings and are capable of flying long distances (Newell and Barber 1913, Markin 1970). In this species, no mating flight occurs (Newell and Barber 1913). Instead, dispersal occurs primarily by budding, where queens and work-

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ers disperse on foot to form new nests (Passera 1994, Keller 1995). The lack of a mating flight and the potential for within-nest mating make it difficult to track the dispersal of Argentine ant reproductives. Because queens do not fly, males are likely to disperse further than queens. In addition, queens and workers can often move freely between neighboring nests (Markin 1970, N. Heller, *unpublished data*), and the local mixing of individuals between nests may influence the colony structure of Argentine ant populations. If queens and workers move only short distances, there may be genetic differences among nests within a unicolonial population.

We used genetic analyses of the structure of Argentine ant nests within an invading population to examine the dispersal dynamics of this species. A number of factors make it difficult to use genetic data to estimate demographic features of invasive populations (Villablanca et al. 1998, Davies et al. 1999). Invading ant populations may be subject to genetic bottlenecks, sometimes leading to greatly reduced genetic variability at neutral loci in the introduced population (Tsutsui et al. 2000, Tsutsui and Case 2001), but sometimes not (Giraud et al. 2002). In addition, the high levels of mixing between nests in unicolonial populations may dilute the genetic signature of nests and may require the use of highly polymorphic markers to detect genetic patterns (Krieger and Keller 1999, Ingram and Palumbi 2002). Finally, traditional methods of measuring genetic structure assume equilibrium dynamics and are based on allele frequencies at individual loci. These methods are likely to underestimate the genetic structure of invading populations and alternative methods of analysis, such as multi-locus genotype assignments, may be better suited to such nonequilibrium populations (Davies et al. 1999, Ingram 2002*b*).

Despite these difficulties, genetic studies of invasive ants have so far provided valuable ecological information on species in native and introduced habitats (Ross and Fletcher 1985, Kaufmann et al. 1992, Ross et al. 1997, Ross and Shoemaker 1997, Krieger and Keller 2000, Tsutsui and Case 2001). For Argentine ants, allozyme and microsatellite studies have demonstrated extremely low nestmate relatedness in introduced European populations (Kaufmann et al. 1992, Krieger and Keller 2000). A comparative study of gene flow patterns between introduced and native populations of this species has shown that considerable structure exists among nests in native habitats, but not in introduced habitats (Tsutsui and Case 2001), suggesting that Argentine ant dispersal is more limited in native habitats. By combining genetic structure analyses with ecological and demographic data from a well-studied invasion, our study extends the use of genetic data to explore the dispersal patterns within an invading population.

We examined a population of Argentine ants that has invaded Jasper Ridge Biological Preserve (JRBP), a 481-ha preserve surrounded by developed land in San Mateo County, California. The invasion of *L. humile* into JRBP from surrounding areas has been monitored since 1993 (Human and Gordon 1996, Sanders et al. 2001). By sampling nests along transects that correspond to time since invasion, we used microsatellite data to measure genetic structure at three spatial scales: gene flow across the entire population, levels of substructure within the population, and connectivity (genetic similarity) between neighboring nests. Our analysis compares traditional F_{ST} statistics based on per locus allele frequency differences and genotype assignment tests based on multi-locus probabilities of identity to determine how the genetic structure of this population reflects the invasion dynamics of a unicolonial species. We addressed the following questions:

1) We examined population-level gene flow patterns to compare the dispersal characteristics of males and queens. Do the dispersal patterns of queens and males differ? Are queens limited to local movement between nests?

2) We examined the genetic substructure within and between subpopulations to determine how nests differ within a unicolonial population. Can the origin of the invasion or the direction of population expansion be determined from genetic data?

3) We examined the genetic structure between neighboring nests to examine connectivity, i.e., local dispersal and the exchange of workers between nests. Does the expansion of nests occur primarily from the edge of the invasion front into previously uninvaded habitat, or do new nests at the front originate from distant nests in areas where Argentine ants have been established for many years? As Argentine ants become established, is there a change in the amount of genetic differentiation among neighboring nests?

Methods

Field site and collection of nests

Argentine ant nests were collected along two transects running from adjacent areas into Jasper Ridge Biological Preserve (JRBP), a 481-ha reserve in San Mateo County, California. Argentine ants have invaded JRBP from the surrounding developed and agricultural areas. The progress of the *L. humile* invasion has been monitored in biannual surveys from 1993 to present (Human et al. 1998, Sanders et al. 2001, N. Heller, *unpublished data*). These survey data were used to determine the duration of Argentine ant occupation at the locations sampled in this study.

Two transects were established that correspond to gradients in time since invasion (Fig. 1, Table 1). Transect 1 spanned 1500 m, originating outside of the JRBP boundary in a surrounding area managed by the Stanford Linear Accelerator Center (SLAC). The linear accelerator building is 3.2 km long and runs perpendicular to the transect. Because the building is located 15

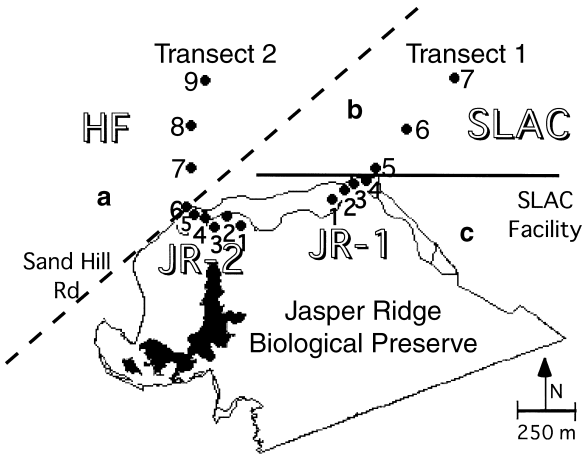

FIG. 1. Map of study site at Jasper Ridge Biological Preserve, northern California. There were seven locations sampled for Transect 1 and nine locations sampled for Transect 2. Each location represents three nests collected 15–20 m apart. Locations are separated by at least 50 m. The four subpopulations, Jasper Ridge (JR-1 and 2), Stanford Linear Accelerator Center (SLAC), and Horse Farm (HF), are designated by block letters. The barriers separating the subpopulations include the SLAC building (solid line, Transect 1) and Sand Hill Road (dashed line, Transect 2). Three possible origins of introduction into JRBP are indicated by bold lowercase letters: **a**, **b**, and **c**.

m below ground and 10 m above ground, it may be a barrier to gene flow between locations 1–4 and locations 5–7. Two putative subpopulations, JR-1 (the area within JRBP adjacent to SLAC) and SLAC (the area to the north of the SLAC facility), were identified for Transect 1. Transect 2 spanned 2000 m and also originated outside of the JRBP boundaries in an adjacent area used as a horse farm (labeled HF). Transect 2 crossed a highway, Sand Hill Road, which may be a potential barrier to gene flow between nest locations 1–6 and locations 7–9. Two putative subpopulations, JR-2 (the area within JRBP adjacent to HF) and HF (the area to the northwest of JR-2, across Sand Hill Rd), were identified for Transect 2.

In the spring of 2001, nests were collected at each of 7 locations for Transect 1 and 9 locations for Transect 2. Locations were mapped via GPS, and distances between locations were calculated from map coordinates. Within each location, three nests were collected at distances 15–20 m apart. *L. humile* nests were collected at midday when the ants aggregrate just below the soil surface. Entire nests were excavated with shovels and placed in a large sampling tray to collect individuals. All nests that had multiple brood chambers at the same nest site were collected as a single nest. Eight adult workers and eight worker pupae were collected from each nest and frozen at −20°C.

Sample extraction and microsatellite analysis

For the genetic analyses, all adult workers and brood were typed from 48 nests, representing 768 individuals in total. Workers and brood (larvae and pupae) were soaked in distilled water for 15–30 min, pulverized, and boiled in 100 μL of a 10% Chelex (Bio-Rad, Hercules, California, USA) solution for 15 min. After boiling, the extraction solutions were centrifuged for 1 min and supernatant was removed. Samples were stored at −20°C. One μL of each sample was used in a 12.5-μL polymerase chain reaction (PCR) reaction.

Seven primer sets were used to genotype each individual at microsatellite loci: Lihu-H, Lihu-O, Lhum-13*, Lhum-35*, Lhum-19*, Lhum-11*, and Lhum-52*. Primer sequences and detailed methods of PCR amplification are described in Ingram and Palumbi (2002) and Ingram (2002*a*). PCR products were run on 5% Sequagel acrylamide gels using fluorescent dyes, and the gels were subsequently analyzed using GENESCAN software (Applied Biosystems-Perkin Elmer 2000).

To correct for possible nonindependence of nestmate genotypes in measures of genetic structure (Ross et al. 1997), a resampling procedure was used to conduct tests of Hardy-Weinberg equilibrium and to estimate *F* statistics (Ingram 2002*a*). A single individual was drawn at random from each nest (with replacement) to create 1000 distributions of independent genotypes. Deviations of genotypes from Hardy-Weinberg equilibrium proportions (HWEP) were tested at each locus for the resampled genotype distributions with a Markov chain approximation suitable for loci with more than four alleles (Guo and Thompson 1992). A global test according to Fisher's method was performed across all loci for each resampled genotype distribution in GENEPOP (WWW version 3.1c [available online][2]; Raymond and Rousset 1995). Significant departures from HWEP were tabulated for each locus and for each resampled population, and these values were evaluated for conformity to HWEP at a significance level of $\alpha = 0.05$.

Genetic analysis

The genetic structure of nests in the JRBP population was estimated with two methods: Weir and Cockerham's (1984) F_{ST} statistics and multi-locus genotyping assignment tests (Paetkau et al. 1995). F_{ST} statistics measure the amount of genetic variation (or differentiation) in a population due to the differences between subpopulations (F_{ST}), inbreeding within a subpopulation (F_{IS}) and inbreeding within an individual (F_{IT}). F_{ST} measures are based on allele frequencies at individual loci and assume equilibrium dynamics (i.e., allele frequencies in Hardy-Weinberg proportions) in the study population. In the present study, F_{ST} measures for isolation by distance and local connectivity were estimated from resampled genotype distributions in GENEPOP. F_{ST} measures for population substructure were estimated using hierarchical analysis (AMOVA [anal-

[2] URL: ⟨http://wbiomed.curtin.edu.au/genepop⟩

TABLE 1. Duration of *L. humile* occupation (in years) at each sample location (see map in Figure 1).

Transect 1	Duration (yr)	Transect 2	Duration (yr)
1	3	1	3
2	8	2	5
3	8	3	6
4	8	4	8
5	…	5	8
6	…	6	8
7	…	7	…
		8	…
		9	…

Notes: Data were estimated from biannual surveys that monitor the progress of the Argentine ant invasion in Jasper Ridge (Human et al. 1988; Sanders et al. 2001). Ellipses indicate unknown duration; location is outside area of Jasper Ridge long-term survey.

ysis of molecular variance]; Excoffier et al. 1992) in ARLEQUIN (version 2.000; Schneider et al. 2000 [available online]).[3] The subpopulations used in this measure include (1) subpopulations of areas within JRBP and adjacent to the preserve, (2) locations, each consisting of three sampled nests, and (3) individual nests.

Multi-locus genotyping analyses use both individual genotypes and population level allele frequencies to calculate the likelihood of drawing a single genotype from potential sources based on the observed allele frequencies in each source (Davies et al. 1999). Probabilities of multi-locus worker genotypes in nests were determined with assignment tests (Paetkau et al. 1995) with a correction to avoid zero allele frequencies = $1/C$, where $C = 1 +$ ploidy $\times$ number of individuals in the population. The assignment test assumes Hardy-Weinberg equilibrium allele frequencies and independent, unlinked loci (Waser and Strobeck 1998). Assignment distances (D_A) between nests within a population were calculated from the proportion of misassigned individuals in the web-based assignment calculator program, Doh (J. Brzustowski, *unpublished program*). Average assignment distance was calculated with the following equation:

$$D_A = (A_{x,y} + A_{y,x})/2$$

where A_{xy} is a measure (based on assignment probabilities) of how much more likely genotypes of individuals sampled in population x are from population x than from population y (Paetkau et al. 1995). For each transect, average pairwise genetic distances were calculated for nests within and between locations and for nests within and between subpopulations.

Isolation by distance and gene flow

Genetic isolation by distance, or viscosity, i.e., the accumulation of local genetic differences under geographically restricted dispersal within a population (Wright 1943, Slatkin 1993), indicates limited dispersal of both sexes. To test for isolation by distance in the JRBP population, a pairwise F_{ST} matrix between nests was calculated from nest-mate brood for the entire population and for each separate transect in GENEPOP. The regressions of pairwise F_{ST} on geographic distance between nests and the regressions of $F_{ST}/(1 - F_{ST})$ on log(geographic distance) (Rousset 1997) were computed, and the Spearman rank correlation coefficients (r) were tested for significance with Mantel tests. When populations exhibit isolation by distance, the slopes of both regressions are positive.

Similar analyses were performed with pairwise assignment distances. For each transect, pairwise matrices of assignment distances were calculated for nests. Significance values of the regressions of assignment distance on log distance were tested with Mantel tests in GENEPOP.

Population substructure

Patterns of population substructure reflect the partitioning of genetic variation in a population and provide a measure of the degree of genetic similarity between regions due to the migration of individuals or to recent common ancestry. Low values ($F_{ST} \approx 0$) indicate that there is little or no genetic differentiation between subpopulations relative to the total variation in the population. For each transect in JRBP, hierarchical measures of F_{ST} values were calculated for nests, locations, and subpopulations from adult workers and brood. The significance of F_{ST} values were tested in ARLEQUIN using a nonparametric permutation approach (Excoffier et al. 1992). The number of migrants (reproductive individuals) between nests was estimated with Slatkin's (1985) Nm values using the private alleles method computed in GENEPOP.

The degree of population substructure was also estimated by calculating pairwise assignment distances (D_A) with multi-locus assignment tests. Like F_{ST} measures, high D_A values indicate little genotypic similarity between subpopulations. By contrast, subpopulations that have high genotypic similarity will have low D_A values. Pairwise assignment distances between locations were calculated from the proportion of misassigned individuals. Average assignment distances between locations within subpopulations were compared to assignment distances of locations in adjacent subpopulations.

Local connectivity

Local connectivity, a measure of genetic similarity among neighboring nests, was estimated for nests within locations. Because connectivity values measured separately for workers and brood were similiar, the samples were pooled for these results. At a local scale, small values of F_{ST} and/or D_A measured among nests represent high levels of genetic similarity for individuals from nearby nests. Large values of F_{ST} and/or D_A

[3] URL: ⟨http://anthro.unige.ch/arlequin⟩

TABLE 2. Number of alleles and expected heterozygosities (H_E) for seven microsatellite loci typed in Argentine ants (n = 768 individuals) from Jasper Ridge Biological Preserve, California.

Transect 1			Transect 2		
Locus	No. alleles	H_E	Locus	No. alleles	H_E
Lhum-11*	8	0.66	Lhum-11*	8	0.70
Lhum-19*	8	0.70	Lhum-19*	7	0.69
Lhum-35*	18	0.80	Lhum-35*	19	0.82
Lhum-52*	7	0.61	Lhum-52*	4	0.50
Lihu-H	6	0.50	Lihu-H	15	0.64
Lihu-O†	4	0.25	Lihu-O	7	0.61
Lihu-T†	4	0.08	Lihu-T†	7	0.14

† Loci that were not used in measures of F_{ST} or assignment tests.

among neighboring nests represent highly differentiated nests and suggest that mixing between nests is limited. To determine whether nests are more closely related to nearby nests than distant nests, we estimated the connectivity between nests by comparing the genetic structure (F_{ST} and D_A) among nests within locations, relative to between locations, for each transect. At each location, average pairwise F_{ST} (or D_A) among nests within a location were compared with average pairwise F_{ST} (or D_A) between these nests and the nests at different locations. If measures of genetic structure are smaller within locations than between them, genetic connectivity is higher among neighboring nests than distant nests.

In order to determine whether the genetic similarity among neighboring nests was associated with the duration of invasion at a particular location within JRBP, D_A values among nests at a particular location were plotted against the time (number of years) since each location had been invaded. Since the values for the independent variable (D_A) did not show any strong deviation from normality, a regression coefficient was calculated for the association of D_A and time since Argentine ant occupation. The duration of Argentine ant invasion for sampled locations within JRBP ranged from three years to at least eight years.

RESULTS

Genetic diversity

Genotypes were determined at seven microsatellite loci for 768 individuals. The number of observed alleles and heterozygosities per locus are given in Table 2. The number of alleles per locus found in the JRBP population ranged from 4–19. Expected heterozygosities (H_E) of the loci ranged from 0.50 to 0.82, indicating that the loci are highly variable markers appropriate for studying fine-scale population structure. Loci with $H_E < 0.50$ (Loci Lihu-O and Lihu-T for Transect 1 and Lihu-T for Transect 2) were not used in measures of F_{ST} or assignment tests.

Results of the Hardy-Weinberg equilibrium tests revealed that two out of seven loci did not conform to HWEP expectations. For Lhum-19* and Lihu-H, nearly one-third of the 1000 resampled genotype distributions were significantly different from expected proportions. These two loci may be more sensitive to subtle population deviations from HWEP (due to high allelic richness or large repeat size) or may be more susceptible to amplification artifacts (stutter bands, inaccurate sizing, etc.). The heterozygote deficiency found in Lihu-H suggests that the high incidence of homozygotes at this locus may be due to a null allele. Over all loci, 24% of the resampled genotype distributions did not conform to HWEP. However, no significant population differences from Hardy-Weinberg expectations were observed when either Lhum-19* or Lihu-H were removed from the analysis. Because the removal of the two loci had little impact on measures of nest and/or population structure, all loci were presumed to be informative neutral markers and were used to infer genetic structure in the study population.

Gene flow and dispersal distances

There was little isolation by distance apparent across the entire population (Spearman's $r = 0.18$, $P = 0.002$) indicating either high levels of gene flow across the population or recent invasion followed by rapid population expansion. Because Argentine ant populations grow primarily by budding, the expansion of the population is expected to follow a stepwise pattern rather than a ''radiation'' model in which all the nests in an area are recently descended from a single ancestral nest (Slatkin 1985). In species with limited gene flow, the signature of isolation by distance, i.e., genetic differentiation among distant nests, is expected to appear across nearby locations after a substantial amount of time (Slatkin 1993). In this study, it is difficult to determine whether the recent colonization of the area or high gene flow across the population leads to the failure to detect a strong pattern of isolation by distance. However, given that assignment tests show evidence of substructure within the population (see *Results: Population substructure*), the low viscosity across the population suggests that Argentine ant dispersal within the preserve is not limited to neighboring nests and may include long-distance dispersal events.

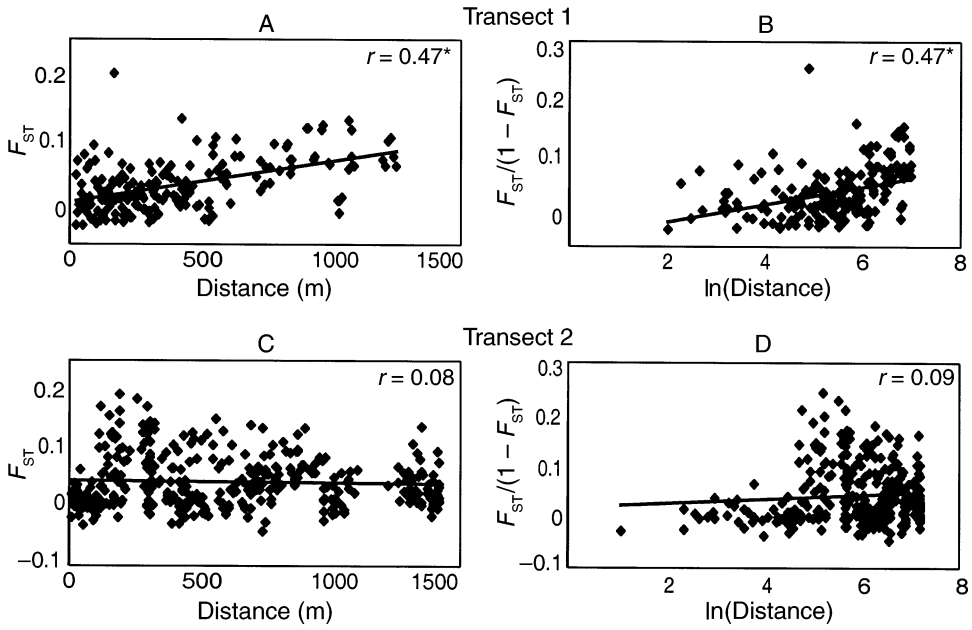

FIG. 2. Isolation by distance with F_{ST} measures of genetic structure. The regressions of pairwise F_{ST} between nests on geographic distance are shown for Transect 1 (A; $r^2 = 0.25$, $r = 0.47$, $P < 0.005$) and Transect 2 (C; $r^2 = 0.00$, $r = 0.08$, $P = 0.14$). The regressions of pairwise measures of $F_{ST}/(1 - F_{ST})$ between nests and log geographic distance (original data were measured in meters before log transformation) are shown for Transect 1 (B; $r^2 = 0.16$, $r = 0.47$, $P < 0.005$) and Transect 2 (D; $r^2 = 0.00$, $r = 0.09$, $P < 0.11$). Asterisks (*) denote significant Spearman's rank correlation (r) at $P < 0.005$.

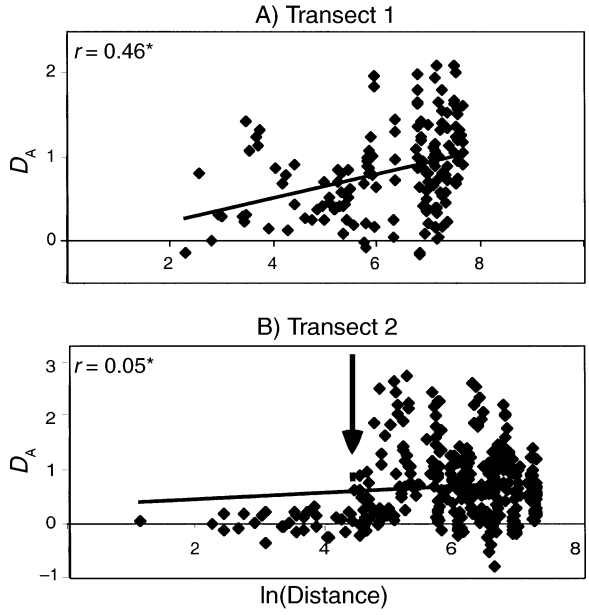

FIG. 3. Isolation by distance with assignment distance measures of genetic structure. The regressions of pairwise D_A between nests on geographic distance are shown for Transect 1 (A; $r^2 = 0.13$, $r = 0.46$, $P < 0.005$) and Transect 2 (B; $r^2 = 0.01$, $r = 0.05$, $P = 0.34$). The arrow in panel (B) shows the point at ~100 m, at which the spread in F_{ST} values for Transect 2 increases. Asterisks (*) denote significant Spearman's rank correlation (r) at $P < 0.005$. Original data were measured in meters before log transformation.

Interestingly, the relationship between pairwise measures of F_{ST} and geographic distance differed between the two transects. Transect 1 showed significant viscosity across the population (Fig. 2A, B), but Transect 2 did not (Fig. 2C, D). For Transect 1, genetic differentiation between pairs of nests increased with geographic distance using both F_{ST} ($r^2 = 0.25$, $r = 0.47$, $P < 0.01$; Fig. 2A) and Rousset's (1997) measure of viscosity, $F_{ST}/1 - F_{ST}$ ($r^2 = 0.16$, $r = 0.47$, $P < 0.01$; Fig. 2B). There was no significant relationship between F_{ST} and geographic distance for either measure for Transect 2 (for F_{ST}, $r^2 = 0.00$, $r = 0.08$, $P = 0.14$; and $F_{ST}/1 - F_{ST}$, $r^2 = 0.09$, $r = 0.09$, $P = 0.11$; Fig. 2C, D). Similar differences between transects were seen when assignment distances were used to measure viscosity ($r^2 = 0.13$, $r = 0.46$, $P < 0.005$ and $r^2 = 0.01$, $r = 0.05$, $P = 0.34$; Fig. 3A, B).

The genetic data for Transect 2 suggest that the JRBP population experiences extensive local dispersal by reproductives with some long distance migration. At small distances, average pairwise genetic differentiation measures (including F_{ST}, $F_{ST}/1 - F_{ST}$, and D_A) were low, with little variance of the estimates. At 75–100 m, the spread in the average pairwise differentiation measures increases. An example can be seen in the spread of D_A values plotted against log(distance) for Transect 2 (Fig. 3B). Nests located approximately <100 m apart are genetically similar and show little structure; F_{ST} or D_A values are all close to zero. The pairwise measures of D_A between nests separated by

TABLE 3. Hierarchical *F* statistics for Transects 1 and 2, and for Transects 1 and 2 combined.

	Hierarchical F_{ST} (AMOVA)				
Transect	Among subpopulations	Among locations	Within locations	Within individuals	*Nm*
1	−0.11	−0.10	−0.01	0.00	5.8
2	−0.06	−0.07	0.01	0.08*	5.1
1 and 2	−0.05	−0.06	0.01	0.03	4

Notes: This analysis partitions genetic variation among subpopulations, among locations, within locations, and within individuals. *Nm* are estimates of migration (number of reproductive individuals between nests per generation) based on Slatkin (1993). The asterisk (*) denotes a significant difference from zero at $\alpha = 0.05$.

distances >100 m are highly variable (see arrow in Fig. 3B), with values as high as $F_{ST} = 0.3$. In Transect 1, the pattern is less clear because the transect crosses between two highly structured subpopulations (JR-1 and SLAC; see *Results; Population substructure*). The pairwise comparisons of genetic structure for this transect reveal a few highly differentiated neighboring nests that correspond to pairs of nests that cross the JR-1/SLAC border.

Population substructure

Estimates of population subdivision using hierarchical measures of F_{ST} are similar in the two transects (Table 3). In general, F_{ST} values were low across nests, locations, and subpopulations and were not significantly different from zero. The low values of F_{ST} indicate high levels of genetic similarity between nests and across transects. Migration estimates (Slatkin's *Nm*) range from ~4–6 reproductives between nests per generation. Inbreeding coefficients, $F_{IT,}$ for Transect 1 nests were also low ($F_{IT} = 0.01$), but Transect 2 had values of $F_{IT} = 0.08$ ($P = 0.05$) within nests. This result suggests that some nonrandom mating with respect to genotypes occurred in nests along this transect, perhaps due to within-nest mating.

Despite the limited isolation by distance across the population, sampled locations are genotypically distinct. If individual genotypes were randomly distributed across a population of nests, assignment probabilities of genotypes within nests would be close to zero. If, instead, nests were completely isolated from other nests and no mixing between nests occurred, high probabilities of genotype assignment to nests (near 100%) would be expected. Due to the mixing between neighboring nests in JBRP, individual ants were assigned to locations for this analysis. The results of the assignment tests (Table 4) show that an average of 50% ± 6% of individuals were correctly assigned to location in Transect 1 (range = 32–72%) and 40% ± 6% of individuals were correctly assigned in Transect 2 (range = 9–68%). For a species with multiple reproductive queens per nest (Krieger and Keller 1999, Ingram

TABLE 4. Genotypic assignments by location for Transect 1 and Transect 2.

	Assigned location								
Source location	1	2	3	4	5	6	7	8	9
Transect 1									
1	27	5	9	0	3	4			
2	7	15	9	5	7	5			
3	9	5	18	2	11	3			
4	6	5	5	18	11	3			
5	0	3	12	0	26	5			
6	4	2	4	0	4	34			
Proportion correct	0.51	0.43	0.32	0.72	0.42	0.63			
Transect 2									
1	15	2	8	0	5	7	4	3	4
2	5	11	9	4	1	9	1	5	3
3	1	1	20	4	5	5	3	7	2
4	1	3	0	42	1	0	1	0	0
5	3	0	8	1	18	8	1	4	5
6	6	1	9	4	8	4	1	11	4
7	2	2	3	2	0	2	28	7	2
8	1	2	9	1	1	8	4	17	5
9	1	2	7	4	6	1	4	7	16
Proportion correct	0.43	0.46	0.27	0.68	0.40	0.09	0.60	0.28	0.39

Notes: The values in the table matrix represent the number of individuals from the source location (rows) that were assigned by genotype to a particular location (columns). The bottom row lists the proportion of individuals correctly assigned to their source location.

TABLE 5. Average genetic assignment distances (D_A) between nests within subpopulations and across subpopulations (e.g., JR–SL) for each transect.

Nest comparison	D_A (±1 SE)	P†
Transect 1		
Within JR	0.46 ± 0.04	<0.001
JR–SL	0.90 ± 0.05	···
Within SL	1.04 ± 0.12	0.14
Transect 2		
Within JR	0.59 ± 0.05	0.02
JR–HF	0.73 ± 0.04	···
Within HF	0.54 ± 0.06	0.01

† P values test differences of D_A within vs. D_A across subpopulations.

TABLE 6. Genetic differentiation among nests within locations and between locations for each transect.

Nest comparison	Within location	Between locations	P
F_{ST} (±1 SE)			
Transect 1	0.01 ± 0.01	0.04 ± 0.01	0.02
Transect 2	0.01 ± 0.01	0.05 ± 0.01	<0.01
D_A (±1 SE)			
Transect 1	0.17 ± 0.09	0.92 ± 0.11	<0.01
Transect 2	0.12 ± 0.04	0.69 ± 0.11	<0.01

Note: Both average pairwise F_{ST} values and average pairwise assignment distances (D_A) among nests are higher between locations than within locations.

2002*a*, *b*) and fluid nest structure, these results show that local genotypic differentiation does exist within this unicolonial population.

Both transects had significant differences in average assignment distances estimated for the subpopulations across the potential gene flow barriers (Table 5). For Transect 1, nests within JRBP had average distances of 0.46 ± 0.04, compared to average distances of 0.90 ± 0.05 between nests in JRBP and the neighboring SLAC subpopulation. For Transect 2, nests had average distances of 0.59 ± 0.05 within JRBP and 0.54 ± 0.06 within the Horse Farm subpopulation. These values were significantly different from the average distance of 0.73 ± 0.04 calculated between the JRBP and Horse Farm subpopulations. Assignment distances between nests in the two JRBP subpopulations (JR-1 and JR-2) were 0.46 ± 0.04.

Connectivity and local mixing between nests

Comparisons of average pairwise F_{ST} estimates between nests reveal significant local subdivision within vs. between locations (Table 6). A similar result was obtained using assignment distances (Table 6). Average pairwise assignment distances calculated for nests within locations were significantly lower than assignment distances between locations.

The regression of average pairwise D_A within locations with time since Argentine ant occupation reveals that connectivity among neighboring nests decreases in both transects as the invasion progresses within JRBP ($r^2 = 0.66$, $F = 13.35$, $P < 0.01$). Fig. 4 shows the relationship of genetic differentiation (D_A) between neighboring nests at locations in JRBP and the age of Argentine ant occupation for the locations for which the age of occupation was known. The higher pairwise assignment distances among nests in areas occupied by Argentine ants for longer periods suggests that the genetic differentiation of neighboring nests increases with time since invasion.

DISCUSSION

Dispersal distances of males and queens

Local movements between nests appear to drive the population genetic patterns of Argentine ants in the Jasper Ridge Biological Preserve. We found that the differentiation between nests within locations is substantially less than between locations, suggesting limited movement between locations. Local dispersal primarily involves the movement of queens on foot (because they don't have wings), but may also include males. If queens were moving only between neighboring nests, pairwise comparisons of nests within the range of queen dispersal would show little or no structure. Our data for Transect 2 allows us to estimate that local dispersal ranges from 0 to ~100 m. All pairwise comparisons of nests <100 m have high genetic similarity or low genetic structure, indicating considerable mixing of individuals between nests within this distance. At distances >100 m, estimates of structure are highly variable, suggesting that mixing among distant nests is less frequent.

The pattern of high local dispersal, at the spatial scale of ~100 m, is not as clear in Transect 1 as in Transect 2. For Transect 1, some nest pairs separated by distances <100 m have large pairwise F_{ST} values, indicating low genetic similarity or high structure between neighboring nests. Interestingly, these nest pairs (see Fig. 2A, B) correspond to nests that flank the SLAC barrier between the two subpopulations, JR-1, and SLAC. Within each subpopulation of Transect 1, all

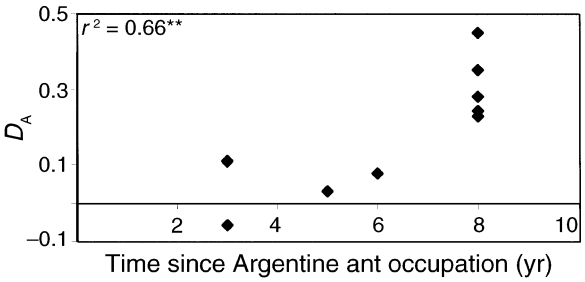

FIG. 4. Average assignment distances between neighboring nests at each location sampled in Jasper Ridge Biological Preserve. The *x*-axis denotes the number of years Argentine ants have been found at each location. The points designated 8 yr represent invasion duration of at least eight years.

**Significant regression coefficient for invasion duration and D_A at $P < 0.01$.

nest pairs separated by distances <100 m do show low F_{ST} values similar to those found in Transect 2. This suggests that within the two subpopulations of Transect 1, there is also considerable mixing of queens and workers at distances <100 m. The weak isolation by distance pattern seen across the entire Transect 1 may be due to the comparison of nests from two separate invasion events (see *Discussion: Structure in an invading Argentine ant population*).

With limited queen dispersal and high rates of intranidal mating, gene flow analyses should show high viscosity or isolation by distance between nests. In the Jasper Ridge Biological Preserve, the overall pattern of gene flow across the population shows little isolation by distance, suggesting that some long distance dispersal is homogenizing allele frequencies across the population. As males are the primary agents of long-distance dispersal, they may play an important role in determining genetic differentiation at spatial scales larger than 100 m.

Newly invading populations also show low genetic viscosity across nests when populations are not yet at genetic equilibrium (Slatkin 1993, Rousset 1997). Argentine ants have been invading JRBP for at least nine years and have been established in the surrounding areas for longer (Sanders et al. 2001), but there may not have been sufficient time for drift to produce measurable genetic differentiation. However, given the evidence of local differentiation with both F_{ST} and assignment distance measures and the fact that nest dispersal occurs via budding, the low genetic viscosity at the scale of this study (0–2000 m) suggests a genetically mixed population. The high gene flow estimates between locations, which are separated by 50–1000 m, indicate that males may be dispersing farther than neighboring nests within locations, which are separated by 10–30 m. It is difficult to estimate from these data precisely how far males are moving, as gene frequencies across a population can be homogenized by multiple stepwise dispersal events or by a few long-distance dispersal events.

Structure in an invading Argentine ant population

Despite high levels of gene flow and the relatively recent introduction of this population, Argentine ant nests in JRBP are not genetically homogeneous. Our genetic analysis distinguished localized effects of dispersal and mixing behavior within a single invasion. The expansion of the population within JRBP has been well documented (Human and Gordon 1996, Sanders et al. 2001; N. Heller, *unpublished data*), but the source of introduction into the preserve is unknown. For this study, the locations of Transect 1 and 2 were established along a gradient of duration of invasion within JRBP. Assuming a continuous expansion into JRBP, we extended the transects into adjacent areas outside the preserve based on the direction of expansion observed inside the reserve. However, the genetic results show true barriers between the subpopulations within JRBP and those adjacent to the preserve, particularly for Transect 1.

The subpopulations separated by the physical barriers, the SLAC facility and Sand Hill Road, do indeed represent populations that are at least partially isolated from their counterparts inside the reserve. For both transects, nests within the two subpopulations in JRBP (JR-1 or JR-2) were genetically more similar to nests in the same subpopulation than were nests in JR-1 compared to nests in SLAC and nests in JR-2 compared to nests in HF. For Transect 1, the two subpopulations (JR-1 and SLAC) are separated by the SLAC facility, a 3.2 km long linear particle accelerator building. Within JRBP, nests along this transect have an average pairwise assignment distance of ~0.5. However, the average D_A for pairs of nests measured between the JR-1 and SLAC subpopulations was nearly twice the assignment distance for pairs of nests measured within JR-1. These results suggest that nests in JRBP are genetically differentiated from nests in the neighboring SLAC area.

Due to the pattern of expansion documented in the surveys, we expected either the SLAC (Transect 1, route b, Fig. 1) or Horse Farm (Transect 2, route a, Fig. 1) subpopulations to be the source of the Jasper Ridge invasion. However, the genetic results reveal that the sources of invasion do not correspond to the directions of our transects. The relatively large genetic differences between the two subpopulations in Transect 1 suggest that the SLAC subpopulation represents a separate introduction of Argentine ants, rather than a source of the JR-1 subpopulation (route b, Fig. 1). Instead, the JR-1 subpopulation may originate from within the preserve, perhaps extending from Transect 2, or may originate outside of the reserve in an unsampled area to the east of JRBP that encompasses an active plant nursery, often a source of insect/ant introductions (route c, Fig. 1).

In Transect 2, however, the assignment distances between subpopulations are more similar to distances within each subpopulation. The road that bisects Transect 2 is more likely to represent a barrier to gene flow within a continuous population, rather than a barrier between two separate introductions. The fact that a road and a building are sufficient barriers to influence the amount of gene flow across the subpopulations suggests that dispersal by walking queens is an important mechanism of local gene flow in this species.

Connectivity between nests

One important question concerning the invasion dynamics of unicolonial ants is whether nest propagules are formed at the edge of an invasion or are carried by ''jump'' dispersal to areas distant from their origin (Suarez et al. 2001). Within JRBP, Argentine ant nests have high genetic similarity to neighboring nests located <20 m away. This result indicates that the ex-

pansion of this population is primarily due to the local budding of new nests from existing nests at the invasion front. In another invading Argentine ant population in Hawaii, nest densities reach a plateau in the center of the population (Ingram 2002*a*). The expansion of the Hawaii population also appears to occur via budding at the invasion front because nests at the center reach carrying capacity densities and new nests are formed at the edge. It is likely that the primary mechanism of local spread in invading populations of Argentine ants is budding from nearby nests.

Within JRBP, the highest level of connectivity or genetic similarity between nests occurs in recently invaded areas. Genetic differentiation among neighboring nests increases as the invaders become established, suggesting that nests in recently invaded areas may exchange individuals more frequently than nests in areas where Argentine ants have persisted for years.

An alternative explanation for the high genetic differentiation among established nests is that there is greater genetic diversity in areas occupied by Argentine ants for a longer time. For example, newly budded nests at the edge of the population could resemble neighboring nests because they share a subset of alleles with the source nests. Among more established nests, the influx of unrelated queens or males over time would lead to an increase in differentiation among neighboring nests in these areas. This argument assumes that migration of males and queens into nests increase genetic differences between neighboring nests, and that there is no local mixing between nests. In the case of the Argentine ant, neither assumption holds. Since queens must migrate on foot from nearby nests, they are not likely to be an important source of genetic variation. Although males do appear to play a role in long distance gene flow, the mixing of individuals between neighboring nests (Markin 1970; N. Heller, *unpublished data*) probably swamps any local genetic differentiation due to males. Therefore, this alternative explanation seems unlikely, and our results suggest that nests in established areas exchange individuals less frequently than nests at the edge of the population.

The negative association of invasion duration and the genetic similarity of nearby nests indicates that extensive local mixing does not occur in more established nests. The amount of exchange of individuals among nests may depend on nest age or environment. High nest densities and habitat saturation affect queen number and nest ecology in an introduced population of Argentine ants in Hawaii (Ingram 2002*a*). The results of the present study suggest that saturation of an invaded environment may also affect the dispersal behavior of reproductives and the exchange of workers between nests. Favorable environments, not yet invaded, with ample food and nesting resources, may promote rapid budding and frequent mixing of individuals among neighboring nests of unicolonial species. As the invasion proceeds, the saturation of the environment may lead to less frequent nest budding and a less fluid social structure with fewer individuals mixing between nests. It would be interesting to investigate whether the pattern of nest differentiation in this study is found in other invasions of unicolonial ants.

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