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Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta*

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Abstract Social insects are among the most successful and damaging of invasive taxa. We studied spatial and temporal variation in two traits, colony genetic structure and worker mass, associated with social insect success in the introduced fire ant *Solenopsis invicta*. Our aim was to determine if changes in social structure occurred over time and if variation in worker size was related to worker genotype. We sampled 1139 workers from five multiple-queen *S. invicta* nests on six dates over a one-year period. The genotypes of workers were determined at ten microsatellite loci and at the selected locus *general protein-9* (*Gp-9*). We found little evidence for genetic differentiation of workers sampled from distinct nests or from different dates at the microsatellite loci. However, worker *Gp-9* genotype frequencies varied among nests and over time. In addition, worker mass was affected by nest-of-origin, sampling date, ploidy level, and *Gp-9* genotype. Our results suggest that large numbers of queens contribute to the production of workers in introduced *S. invicta* nests throughout the year. Colony boundaries are semi-permeable, although the among-nest variation in *Gp-9* genotype frequencies and worker mass does suggest that boundaries are present. In addition, selection operating on *Gp-9* genotype depends on nest environment. Finally, worker mass is affected by both endogenous and exogenous factors in *S. invicta*. Overall, our data suggests that the key traits of colony social structure and worker size reflect the effects of variable selection in invasive social insects.

Keywords Insect pest · Formicidae · Genetic structure · Invasive ant · Microsatellites · Polygyny · Relatedness · Social insects

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Introduction

Invading species disrupt the organization of natural environments, drive out endemic taxa, and cause billions of dollars in damages each year (Williamson 1996; Pimentel et al. 2000). Social insects are particularly devastating and successful invaders. A variety of introduced termites, ants, bees, and wasps cause considerable ecological and economic damage in their introduced habitats (Vinson 1986; Williams 1994; Moller 1996). Indeed, seven of the 17 most detrimental invasive land invertebrates are social insects (Lowe et al. 2000).

The tremendous success of invasive social insects partly stems from the ecological advantages

derived from their social systems. The cooperative societies displayed by social insects provide great flexibility and readily change to allow invading social insects to operate efficiently in new habitats (Moller 1996). In fact, the social systems of many invasive social insects differ in their native and introduced habitats (Ross et al. 1996; Chapman and Bourke 2001; Holway et al. 2002; Tsutsui and Suarez 2003). In addition, changes in social structure in new habitats affect the genetic structure of populations. Such variation may alter the nature of kin selection, potentially leading to changes in the behavior of workers, the skew in reproductive success, and the sex ratio produced by colonies (Bourke and Franks 1995; Crozier and Pamilo 1996; Ross 2001).

The fire ant *Solenopsis invicta* is a particularly important and successful social insect invader. *S. invicta* originates from South America, but has been introduced into several countries (Callcott and Collins 1996; Tschinkel 1998; Davis et al. 2001; Henshaw et al. 2005). *S. invicta* was introduced to the US ca. 1935. It rapidly spread throughout the southeast of the country and now occupies ~100 million hectares of land. In the US, *S. invicta* has emerged as a major pest, and negatively affects native species, agriculture, the environment, and human health (Vinson 1997).

Like other invasive social insects, *S. invicta* displays variation in social structure, which may be associated with its success. In both introduced and native populations, colonies are headed by either one queen (monogyny) or many queens (polygyny) (*S. invicta* social biology reviewed by Ross and Keller 1995; Ross et al. 1996; Tschinkel 1998). The two social forms differ in a wide array of important characteristics including the size of colony members, number of sexuals produced, fecundity of queens, mode of colony founding, dispersal ability of sexuals, and relatedness of nestmates. In addition, the social system of native populations differs from introduced populations. For example, in native populations, polygyne nests are characterized by low numbers of queens, strong colony boundaries, and high nestmate relatedness. In contrast, in introduced populations, polygyne colonies display high queen numbers, weak colony boundaries, and low nestmate relatedness.

Another remarkable aspect of *S. invicta* is that the two social forms have a genetic basis. Specifically, variation at the locus *general protein-9* (*Gp-9*) is strongly associated with queen number (Ross 1997; Krieger and Ross 2002). Monogyne ants always possess the *B* allele at this locus, whereas polygyne ants possess both the *B* and *b* (or *b*-like) alleles. In introduced populations, reproductive polygyne queens are always heterozygous, *Bb*, while polygyne prereproductive queens (gynes) and workers display all three genotypes (*BB*, *Bb*, and *bb*). Of additional importance, gynes and workers of different *Gp-9* genotype differ in mass (Goodisman et al. 1999; Keller and Ross 1999; DeHeer 2002). Gynes of distinct genotypes also show variation in mating and reproductive behaviors (DeHeer et al. 1999, 2002; Goodisman et al. 2000a), and workers of alternate *Gp-9* genotype differ in how they interact with gynes (Keller and Ross 1998; Ross and Keller 1998).

The frequencies of the *Gp-9* genotypes in polygyne *S. invicta* gynes and workers differ from simple expectations based on random association of alleles (Ross 1997; Goodisman et al. 2000b). The unusual genotype frequencies in gynes apparently arise from selection operating differentially on the genotypic classes. *BB* gynes are killed by workers as they mature and *bb* gynes apparently suffer high mortality related to endogenous factors (Keller and Ross 1998, 1999; Ross and Keller 1998). The nature of selection operating on workers is unclear, but may be related to the size differences among the genotypic classes (Goodisman et al. 1999).

The primary goal of this study is to understand changes in genetic and social structure of single polygyne *S. invicta* colonies over time. Variation in social structure is relevant to the invasive biology of ants, because changes in social structure, such as the breakdown of colony boundaries, are associated with successful ant invasions (Holway et al. 2002). A secondary goal of this study is to understand factors affecting worker size in *S. invicta*. Worker size is believed to be under selection in ants (Oster and Wilson 1978). In addition, worker size may be an important factor associated with successful invasive ants, which tend to be smaller relative to their native counterparts (McGlynn 1999). In this study we

disentangle the effects of environment and genotype on worker size in *S. invicta* and consider how such effects might be associated with the success of invasive *S. invicta*.

Materials and methods

Sample collection

We collected *S. invicta* workers from two sites approximately 5 km apart in Walton County, Georgia, United States. Thirty-nine and seven polygyne nests were originally sampled from sites 1 and 2, respectively. All nests were marked with metal pit tags so they could be located on subsequent sampling days. *S. invicta* workers were collected from nests on five distinct dates spanning approximately one year. The dates (and number of days from first sampling) were February 21 (1), March 21 (29), May 6 (75), June 12 (112), September 20 (212) of 2004, and March 19 of 2005 (392). Workers were collected from nests by inserting a 15 ml conical tube lined with talc directly into the top of the nest and allowing workers to fall into the tube. Workers were then preserved in 95% ethanol for subsequent analysis.

Over the course of sampling, some colonies apparently abandoned their original nests, other colonies died off altogether, and still others could not be found again. Consequently, the numbers of nests from which workers were sampled declined over time. By the time sampling ended, nine nests from site 1 and three nests from site 2 could not be located. Moreover, ants were collected from only 20 of 30 and one of four nests that could be located from sites 1 and 2, respectively.

Laboratory methods

We ultimately focused our analyses on five nests from site 1 (hereafter nests numbered 77, 92, 121, 123, and 127). We chose to study variation in relatively few nests so that we could analyze many workers from each nest on each sampling date. Workers from these nests were chosen for analysis because the nests (1) did not move, (2) were separated by a range of distances (2–118 m) thereby facilitating analysis of spatial genetic patterns, and (3) contained large numbers of workers on all six sampling dates. A random subset of workers from each collection was selected for analysis. The mass of each worker was determined to the nearest hundredth of a milligram. Genomic DNA was then extracted from each ant (Crozier et al. 1999).

We obtained the multilocus genotype of workers at the ten microsatellite loci M-II, M-III, M-IV (Chen et al. 2003), *Sol-11*, *Sol-18*, *Sol-20*, *Sol-49*, *Sol-42*, *Sol-55* (Krieger and Keller 1997), and *Sol-6* Modified (Cahan and Vinson 2003). The forward PCR primers for each of these loci were endlabeled with the fluorescent dyes 6-FAM, HEX, or NED. PCR products were visualized on an Applied Biosystems PRISM® 3100 Genetic Analyzer.

We also genotyped workers at the locus *Gp-9*. We used a modification of the method of Valles and Porter (2003), which is designed to detect the presence of the two alleles found in the US using allele-specific (*B* or *b*) primers. We multiplexed the *B*-specific primers (26BS and 16BAS) or the *b*-specific primers (24bS and 25bAS) with primers for microsatellite locus M-II. Multiplexing the *Gp-9* allele-specific primers with microsatellite primers ensured that a lack of band for a particular *Gp-9* allele indicated that the individual did not possess that allele, and not that the PCR failed.

PCRs for detecting *Gp-9* genotypes were conducted in 15 μl final volume containing 1 μl genomic DNA and ~1 U Taq DNA polymerase, and a final concentration of 200 μM dNTPs, 0.2 μM of the forward and reverse M-II PCR primers, 0.8 μM of the forward and reverse *Gp-9* PCR primers (either 26BS and 16BAS or 24bS and 25bAS), and 1× PCR buffer. The PCR cycling profiles began with an initial denaturation at 94°C for 2 min, and then proceeded with 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. All PCRs were run in duplicate to ensure consistent results and the products were run on 2.5% agarose gels to distinguish the bands. To confirm the robustness of the method, we analyzed the genotypes of 16 monogyne workers. As expected, all 16 monogyne workers displayed the *B* amplification product and none displayed the *b* amplification product. In contrast, polygyne workers

displayed all three possible genotypes, suggesting that the assay was functional.

Statistical analyses

A fraction of polygyne *S. invicta* workers in the US are triploid (Krieger et al. 1999). We identified triploid workers in this study, because they displayed three alleles at one or more of the microsatellite loci. To more readily analyze the microsatellite data, we assigned triploid workers two of the three alleles they possessed by randomly eliminating one of the three alleles. This method does not affect the analyses provided that nuclear alleles associate at random within workers, as appears to be the case (see below).

We used the program GENEPOP 4.2 to study the distribution of genetic variation within our population (Raymond and Rousset 1995). The magnitude of nonrandom associations of alleles within individuals was quantified by the statistic F_{IS}. A probability test was used to determine the significance of deviations from Hardy–Weinberg expectations at each locus (Rousset and Raymond 1995). In addition, exact tests were used to test for genotypic disequilibrium between pairs of loci. Genetic differentiation among nests or within nests among time points was measured by Wright's *F*-statistics (Weir and Cockerham 1984) as implemented by the program GDA (Lewis and Zaykin 2000). The resulting nonrandom associations of alleles due to genetic differentiation of time points within nests and between nests within the population were quantified with the statistics θ_T and θ_N, respectively. The 95% confidence intervals for the statistics were determined by bootstrapping over loci 1,000 times.

We also estimated the relatedness of nestmates using the program RELATEDNESS 4.2 (Queller and Goodnight 1989). We combined all individuals from all times within nests to obtain an overall estimate of nestmate relatedness. We also considered the nest as 'deme' and each time sample within nest as the 'group' to obtain an estimate of relatedness of nestmates sampled from different times. Standard errors for estimates were obtained by jackknifing over nests. Estimates for the effective number of queens contributing to offspring were obtained by taking the estimate of relatedness (r) and evaluating the formula $q_e = 3/4r$.

We next estimated F for workers sampled from all pairs of time points within nests. We then conducted Mantel tests using GENEPOP to determine if there was a relationship between sampling date and genetic differentiation. In addition, we tested for differences in the frequencies of the three *Gp-9* genotypes across nests and over time using nominal logistic regression. Significance of effects was determined by a likelihood ratio test.

Finally, we used a four-way ANOVA including the fixed effects of nest, sampling date, *Gp-9* genotype, and ploidy level, in conjunction with all two-way interactions, to determine if any factors were associated with significant variation in *S. invicta* worker mass. Because the residuals of the ANOVA on the raw mass data were not normally distributed, we also conducted ANOVA on the ranks of mass using Scheirer–Ray–Hare extension of the Kruskal–Wallis test (Sokal and Rohlf 1995).

Results

Variability and associations of markers

We obtained the multilocus genotypes of 1139 workers that were sampled from five nests on six different days (37.97 ± 9.36 workers per sample, mean ±SD). The estimates of expected heterozygosities at the microsatellites and at *Gp-9* (Table 1) were similar to estimates previously obtained using these markers (Krieger and Keller 1997; Chen et al. 2003). Estimates of F_{IS} for most microsatellite loci were low and associated probability tests typically showed no significant deviations from expectations of random associations of alleles (Table 1). However, two microsatellite loci, *Sol-42* and *Sol-55*, showed positive estimates of F_{IS} and highly significant deviations from Hardy–Weinberg expectations. In addition, we also observed a significantly negative estimate of F_{IS} at the locus *Gp-9*.

Results of exact tests of genotypic disequilibrium revealed that genotypes of most loci were independent of those at other loci. Indeed, there

Table 1 Number of alleles (N_A), expected heterozygosity (H_e), estimate of F_{IS}, and probability of locus deviating from Hardy–Weinberg expectations (P) for each of 11 loci in introduced *S. invicta*

Locus	N_A	H_e	F_{IS}	P
M-II	5	0.618	0.031	0.299
M-III	4	0.577	–0.104	0.051
M-IV	5	0.548	0.025	0.293
Sol-6 Mod	7	0.456	–0.009	0.096
Sol-11	6	0.725	–0.039	0.883
Sol-18	2	0.095	–0.049	0.999
Sol-20	7	0.696	–0.013	0.989
Sol-42	**4**	**0.691**	**0.259**	**<0.001**
Sol-49	8	0.696	0.008	0.723
Sol-55	**7**	**0.572**	**0.053**	**<0.001**
Gp-9	**2**	**0.487**	**–0.368**	**<0.001**

Loci that showed significant deviations from Hardy–Weinberg equilibrium as determined by probability tests are given in bold

was no significant genotypic disequilibrium between any pair of microsatellite loci after Bonferroni corrections. However, we did detect significant genotypic disequilibrium after Bonferroni adjustments between the selected locus *Gp-9* and the microsatellite locus *Sol-55* ($P < 0.0001$ for overall test). These two loci showed significant to moderately significant genotypic disequilibrium in several samples ($P < 0.1$ in 16 of the 30 samples).

Finally, we identified 70 workers as triploid based on their multilocus genotype. This frequency is significantly below that found in a previous study of triploidy in introduced *S. invicta* ($G_1 = 19.18$; $P < 0.0001$; Krieger et al. 1999). In addition, we found that the frequency of triploidy differed among nests ($G_4 = 6.80$; $P = 0.0091$).

Genetic structure

We analyzed the microsatellite data to detect the presence of genetic structure among nests or over time within nests. For these analyses, we used the reduced dataset that excluded information from the locus *Gp-9*, which is apparently under selection. In addition, we excluded *Sol-42* and *Sol-55* from our analysis, because they showed significant deviations from Hardy–Weinberg expectations.

Our analysis of the data revealed little evidence of structuring of alleles within individuals (f), among time points within nests (θ_T), or between nests within the total population (θ_N). Our estimates (and associated 95% confidence intervals) for these statistics were $f = –0.020$ (–0.117 to 0.065), $\theta_T = 0.004$ (0.001 to 0.007), and $\theta_N = 0.001$ (–0.001 to 0.003), with an overall estimate of $F = –0.016$ (–0.111 to 0.067).

We found that the relatedness of nestmate *S. invicta* workers in the five nests, $r = 0.004 \pm 0.019$, did not differ significantly from zero. From these data the estimate of the effective queen number was $q_e = 187.5$, but the 95% confidence limits for effective queen number ranged from 17.85 to infinity. In addition, the relatedness of workers sampled on distinct sampling dates from within nests, $r = 0.004 \pm 0.015$, did not differ significantly from zero.

We were next interested in determining if workers sampled from the same nests on different dates showed consistent increases in variation of allele frequencies over time, as might be expected if reproductive turnover occurred. In this case, we would expect a positive correlation between genetic differentiation of samples within nests and sampling date. However, three of the five correlations between these variables were negative and none were significantly different from zero (Table 2). Consequently, our microsatellite data revealed little evidence of genetic differentiation of workers sampled from distinct nests or sampled on different dates from the same nests.

Finally, we found significant variation in *Gp-9* genotype frequencies across nests and over time ($\chi^2_{58} = 124.56$; $P < 0.0001$). The significant results were due to effects of nest ($\chi^2_8 = 27.24$; $P = 0.0006$), sampling date ($\chi^2_{10} = 31.64$;

Table 2 Spearman's correlation (r_S) between genetic differentiation of *S. invicta* workers sampled on different dates and days between sampling

Nest	Correlation (r_S)	P
77	–0.196	0.7190
92	–0.268	0.7543
121	0.509	0.0780
123	0.296	0.2199
127	–0.146	0.7087

The significance of each correlation was determined by a Mantel test

$P = 0.0005$), and the interaction between nest and date ($\chi^2_{40} = 67.18$; $P = 0.0045$). In general, the frequency of heterozygotes was always highest within nests, but the frequency of homozygous types fluctuated (Fig. 1).

Variation in worker mass

Our full models considering the effects of nest, sampling date, *Gp-9* genotype, and ploidy on mass and rank-of-mass yielded highly significant

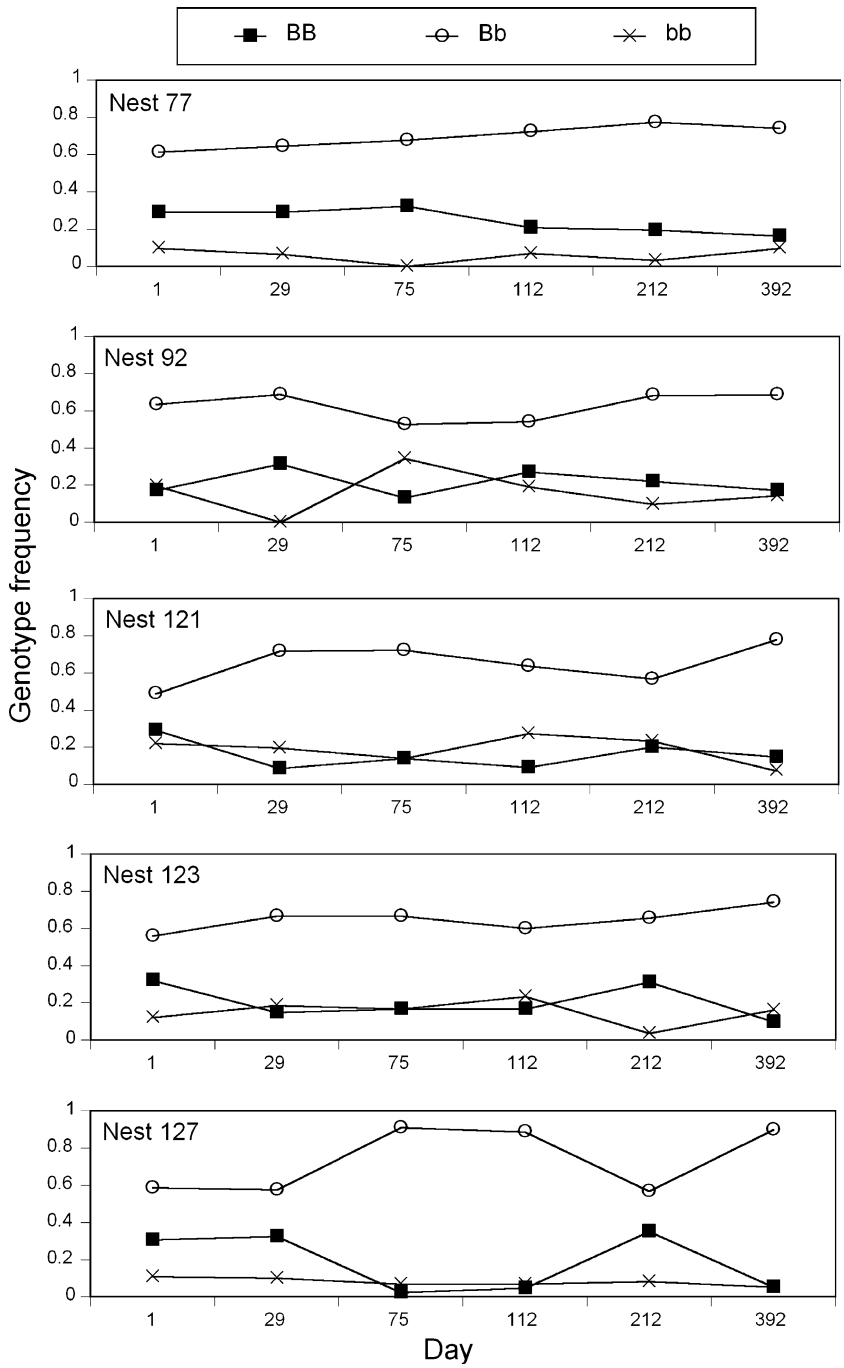

Fig. 1 Frequency of *Gp-9* genotypes in introduced *S. invicta* workers sampled from five nests on six distinct days

results ($F_{61,\ 961}$ = 3.81 and 3.35 for raw mass and rank-of-mass, respectively; $P < 0.0001$). However, none of the main effects accounted for significant variation in the full models. Rather, the significant effects arose from the interactions between nest and date ($P < 0.0001$), as well as nest and *Gp-9* genotype ($P = 0.0015$).

To increase the power of our test, we reran our analyses without the nonsignificant two-way interaction terms. Once again, the ANOVA explained significant variation in the data ($F_{40,982}$ = 5.36 and 4.70 for raw mass and rank-of-mass, respectively; $P < 0.0001$). However, in contrast to the full model, the reduced model yielded significant effects of all main factors, as well as the two-way interactions (Table 3). The models based on the response variable mass and rank-of-mass gave similar results with the exception of the effect of *Gp-9* genotype. The analysis of the raw mass data suggested that *Gp-9* genotype had no significant effect on worker mass, whereas the analysis of rank-of-mass data indicated a highly significant effect of *Gp-9* genotype (Table 3).

We compared the mean values of our factors in the reduced model using Tukey's honestly significant differences (Sokal and Rohlf 1995). We found that workers from nest 92 or sampled on day 75 were significantly more massive than workers sampled from most other nests or dates (Table 4). In addition, triploid workers were significantly more massive than diploid workers. Also, our analyses suggested that workers of *Gp-9* genotype *BB* were significantly less massive than *Bb* or *bb* workers, when rank-of-mass was considered. We also note that the variance in mass of the *BB* workers was significantly larger than for *Bb* and *bb* workers (O'Brien's test; $F_{2,\ 1020}$ = 5.16; $P = 0.0059$).

Table 3 Results of ANOVA of the effects of nest, sampling date, *Gp-9* genotype, and ploidy on workers mass (and rank-of-worker mass) in introduced *S. invicta*

Source	DF	*F*
Nest	4 (4)	14.37*** (6.66***)
Date	5 (5)	2.41* (2.63*)
Gp-9 *genotype*	2 (2)	1.09 (7.13***)
Ploidy	1 (1)	8.33** (11.38***)
Nest*Time	20 (20)	3.61*** (3.39***)
Nest*Gp-9 genotype	8 (8)	4.01*** (3.56***)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Discussion

Invasive social insects damage natural ecosystems (Vinson 1986; Williams 1994; Moller 1996; Lowe et al. 2000). A better understanding of the mechanisms by which invasive social insects operate requires studies of social insect populations over time. Such studies can reveal how social insects come to dominate invaded habitats and reach pest levels (e.g., Greenberg et al. 1992; Clapperton et al. 1994; Ingram and Gordon 2003; Pinto et al. 2005).

The purpose of this study was to investigate changes over space and time in genetic structure and worker size in the invasive fire ant *S. invicta*. Our results indicate that polygyne colonies show little variation at neutral nuclear markers among nests or over the time span investigated. However, variation on traits that were under selection, including genotype at the locus *Gp-9* and worker mass, did show variation over the course of this study.

Nest extinction

We initiated this study by sampling workers from 48 polygyne nests from two sites. By the end of the study approximately one year later, only 21 of these nests still contained ants. The loss of some nests was expected. *S. invicta* colonies may move from one nesting location to another (Adams and Tschinkel 2001). In this study, we did notice patterns consistent with colony movement, such as when a new nest would be found in proximity (<1 m) to an abandoned nest. In addition, established colonies may die off, particularly during cold periods (Callcott et al. 2000; Adams and Tschinkel 2001). We also found evidence of colony mortality in our study. In these cases, once-active nests were found to be uninhabited on a particular sampling date and no active nests were found in the vicinity.

Although we expected the loss of a few colonies, we were somewhat surprised that almost all

Table 4 Mean, standard deviation, and range in *S. invicta* worker mass sampled from five nests, six time points, three *Gp-9* genotypes, and two ploidy classes

Category	Level	Mean ±SD (mg)	Range (mg)	Significance
Nest	77	0.614 ± 0.471	0.04–2.98	B (AB)
	92	0.848 ± 0.704	0.03–3.37	A (A)
	121	0.507 ± 0.322	0.09–1.90	B (B)
	123	0.615 ± 0.458	0.09–2.41	B (B)
	127	0.702 ± 0.553	0.07–2.66	B (B)
Sampling day	1—Feb 21 2004	0.595 ± 0.460	0.12–2.61	A (AB)
	29—Mar 21 2004	0.630 ± 0.555	0.03–3.06	A (AB)
	75—May 6 2004	0.761 ± 0.585	0.09–3.02	A (A)
	112—June 12 2004	0.626 ± 0.514	0.04–2.66	A (B)
	212—Sep 20 2004	0.714 ± 0.614	0.15–3.37	A (AB)
	392—Mar 19 2005	0.668 ± 0.476	0.17–2.45	A (AB)
Gp-9 genotype	*BB*	0.623 ± 0.622	0.04–3.37	A (B)
	Bb	0.715 ± 0.541	0.09–3.06	A (A)
	Bb	0.612 ± 0.376	0.07–2.05	A (A)
Ploidy	Diploid	0.654 ± 0.529	0.04–3.37	B (B)
	Triploid	0.863 ± 0.639	0.03–3.02	A (A)

Levels within categories denoted by distinct letters differed significantly with the response variable of raw mass (rank-of-mass)

colonies from one site and about half of the colonies from the other site died off during our study. New polygyne *S. invicta* colonies readily arise through budding from existing colonies (Vargo and Porter 1989). Moreover, DeHeer (2002) recently suggested that *Gp-9* genotype *Bb* queens are potentially capable of establishing new polygyne nests independently. Thus we expected that colonies that died over the winter would be replaced in the summer months.

Our observations on nest extinction may be of relevance to understanding the invasive biology of *S. invicta*. For example, the changes that we observed at these two sites may signal larger trends in the population biology of *S. invicta* in this area. It is conceivable that after years of unfettered expansion, *S. invicta* populations have begun to moderate, perhaps due to the evolved responses of interspecific competitors. However, such speculation can only be addressed through more widespread long-term studies of established *S. invicta* populations.

Spatial and temporal genetic structure at neutral loci

One of the purposes of this study was to detect variation in the social structure of polygyne *S. invicta* colonies. Our estimates of relatedness among spatially separated nests revealed no significant differentiation among workers from distinct nests. In addition, estimates of inbreeding (F_{IS}) were not significantly different from zero. These results were expected and consistent with previous studies in introduced polygyne *S. invicta*, which have demonstrated that individuals sampled from the same nests are statistically unrelated (Ross et al. 1996; Goodisman and Ross 1997, 1998; Chen et al. 2003).

The primary purpose of this study was to understand patterns of temporal genetic structure in polygyne *S. invicta*. Our data indicated that *S. invicta* workers sampled from distinct nests on different days over the course of a year did not differ genetically. Moreover, there was no relationship between the genetic differentiation of workers and the amount of time separating when they were sampled (Table 2). Consequently, polygyne *S. invicta* colonies displayed no significant changes in social structure over the course of this investigation.

There are several potential explanations for this result. First, it is possible that workers that we sampled at the end of our experiment were from the same cohort as the workers sampled at the beginning. However, we view this explanation

as unlikely. The lifespan of small *S. invicta* workers is only 1–3 months when temperatures reach relatively high levels (e.g., 30°C) (Calabi and Porter 1989). Consequently, many workers that were sampled one year apart probably belonged to different cohorts. A second possibility is that the workers sampled at the beginning and end of our sampling period were of different cohorts but produced by the same set of queens. This explanation may partly account for our results, because estimates of longevity for polygyne *S. invicta* queens are on the order of one year (Ross 1988; Goodisman and Ross 1999). Finally, it is likely that workers within polygyne *S. invicta* nests are produced by so many queens that it is difficult to differentiate a cohort of workers produced by one set of queens from a cohort produced by a different set. Indeed, the estimate of the effective number of queens in our study equaled 187.5. Therefore, changes in which queens were reproducing within nests might well go undetected.

Our results demonstrate that aspects of colony social structure, such as queen number and worker relatedness, related to the success of invasive ants do not vary seasonally in introduced *S. invicta*. Consequently, the invasive patterns of *S. invicta* are not likely to be affected by changes in the social biology of the ant. Instead, seasonal variation in the rate of invasion of *S. invicta* results directly or indirectly from abiotic factors such as temperature and rainfall.

Previous studies that have utilized repeated sampling of social insect nests to monitor changes in genetic structure generally found that social systems were stable over short periods of time. For example, Chapuisat et al. (2004) detected little evidence for variation in social structure in monogyne and polygyne nests of the ant *Formica selysi*. In addition, DeHeer and Vargo (2004) found that most *Reticulitermes flavipes* termite colonies maintained their social structure over the course of a two-year period, although infrequent colony fusion did occur. Ross (1986) also failed to find evidence of variation in social structure within *Vespula* wasp colonies sampled over the course of a season, indicating that male contributions to workers did not change. This was also the case in the honeybee *Apis mellifera*, where evidence for changing social structure due to variation in sperm use by queens is minimal (references by Schluns et al. 2004).

Overall, our results are consistent with the view that introduced polygyne *S. invicta* forms unicolonial populations, where colony boundaries are semi-permeable (Bourke and Franks 1995; Crozier and Pamilo 1996). The spatial genetic structure displayed by polygyne *S. invicta* thus reflects some degree of movement of individuals among nests. We note, however, that previously discovered correlations between the number of queens per nest and worker mass (Goodisman and Ross 1996) and the number of queens per nest and worker relatedness (Ross 1993), in addition to the observed among-nest variation in *Gp-9* genotype frequencies and worker size discovered in this study, suggest that transient colony boundaries do exist in introduced *S. invicta*. Regardless, many invasive ants display weak colony boundaries and it has been suggested that this social system allows invasive species to reach high densities and dominate habitats (Holway et al. 2002). This appears to be the case with polygyne *S. invicta*, which reaches higher density than the monogyne form in introduced populations (Macom and Porter 1996).

Spatial and temporal genetic structure at the selected locus G-9

Variation at *Gp-9* is strongly associated with queen number in *S. invicta* (Krieger and Ross 2002). For example, all polygyne *S. invicta* queens are heterozygous at this locus. Therefore, the frequency of heterozygous polygyne workers should equal 0.5, regardless of the genotype (*B* or *b*) of the queen's haploid male mate (Goodisman et al. 2000b). However, we found that the frequency of heterozygous workers, 0.674, significantly exceeded 0.5, in accord with findings from previous studies (Ross 1997; Goodisman and Ross 1999; Goodisman et al. 2000a; Henshaw et al. 2005). Consequently, our data suggest that selection operates on *Gp-9*, or on a locus closely linked to *Gp-9*, in *S. invicta* workers. Specifically, heterozygous workers enjoy viability advantages relative to homozygous *BB* and *bb* workers.

The genotype frequencies found in this study differed somewhat from those in previous studies in this population. Specifically, we found a substantially higher percentage of *bb* workers than previous investigations (Ross 1997; Goodisman et al. 1999, 2000b). At least some of this variation may result from biases in the estimation of allele frequencies in earlier studies. *Gp-9* genotype was previously determined using allozyme electrophoresis (DeHeer et al. 1999). However, it may be difficult to score *Gp-9* genotype using allozyme electrophoresis in small workers because insufficient gene product is present. This creates a potential bias in estimating allele frequencies in workers, because *Gp-9* genotype is associated with worker size in *S. invicta* (Goodisman et al. 1999; this study).

Differences in genotype frequencies observed between this and previous studies may also result from variation in selection on *Gp-9* genotype in workers. For example, we found significant variation in the frequency of *Gp-9* genotypes among nests and over time (Fig. 1). Some nests (e.g., 77) tended to display relatively stable genotype frequencies throughout the year. In contrast, other nests (e.g., 92 and 127) displayed erratic changes in *Gp-9* genotype frequencies. Although heterozygous workers always predominated, the proportions of *BB* and *bb* workers varied considerably. Overall there was no consistent pattern to the changes in homozygous genotype frequencies either within or between nests.

Notably, variation in *Gp-9* genotype frequency of workers or gynes over space or between laboratory- and field-reared individuals has also been discovered in previous studies (DeHeer et al. 1999; Goodisman et al. 1999, 2000a). These results, coupled with the fact that individuals sampled from distinct nests do not differ genetically at neutral loci, suggest that nest environment may affect selection operating on this locus.

Finally, we found an unusual linkage relationship between *Gp-9* and the microsatellite locus Sol-55. The genotypic association is particularly surprising because *Gp-9* and Sol-55 displayed a significant excess, and deficit, of heterozygotes, respectively. It is possible that the genotypic association that we detected is spurious and results from the vagaries of sampling in this study. On the other hand, the association between the loci may also arise because of physical linkage of Sol-55 with *Gp-9* in combination with complex forms of selection acting on the loci. Indeed, other loci linked to *Gp-9* have been uncovered in *S. invicta* (Keller and Ross 1999) and *Gp-9* may reside in a large region of linked loci associated with the many phenotypic effects associated with this locus in polygyne *S. invicta* (Ross and Keller 1998; Krieger and Ross 2002).

Spatial and temporal variation in worker mass

Our analyses revealed that variation in worker mass was associated with nest-of-origin, sampling date, ploidy, and *Gp-9* genotype. Previous studies revealed that mean worker mass decreases with increasing queen number in polygyne *S. invicta* nests (Goodisman and Ross 1996). Consequently, it is possible that the nests sampled in this study differed in queen number, and that queen number was lower in nest 92 which housed relatively more massive workers (Table 4). Nest 92 was found on the periphery of this sampling site. The location of the nest may be associated with the observed variation in worker mass. In the invasive Argentine ant, *Linepithema humile*, nests on the advancing edge of populations contain fewer queens than those in the center (Ingram 2002). It is possible that *S. invicta* nests on the periphery of polygyne populations also contain fewer queens than those in the center. If this were the case, it would explain the observation that workers from the nest sampled on the edge of our site were more massive than those sampled from the interior.

We discovered weak evidence for variation in the mass of workers sampled from distinct times. Workers sampled on May 6, 2004 were significantly more massive than those sampled from June 12, 2004 when rank-of-mass was considered as the response variable (Table 4). We expected that workers sampled in the summer months would be smaller than those sampled during the winter. Such a relationship would arise if small workers suffered relatively higher mortality during the colder winter days. Indeed, such a relationship has been discovered in introduced

monogyne (Tschinkel 1993) and polygyne (Greenberg et al. 1992) *S. invicta*. Thus our results are in general agreement with these previous studies and suggest that worker size is subject to seasonal selection.

The third factor that we found to affect *S. invicta* worker body mass was ploidy. Typically, *S. invicta* workers are diploid, as would normally be the case for female haplodiploid insects. However, a small percentage of polygyne *S. invicta* workers in introduced populations are triploid. Triploid workers likely originate from matings between normal diploid females and abnormal diploid males (Ross and Fletcher 1985; Krieger et al. 1999). The frequency of triploid workers in this study (6.1%) fell significantly below that found in the previous study of polygyne *S. invicta* (12.5%, Krieger et al. 1999). This may reflect the fact that workers were sampled nonrandomly in the earlier investigation, which included a high percentage of 'large' workers. In fact, if we consider only the frequency of triploidy among 'small' workers from Krieger et al. (1999) then the estimates do not differ between the two studies ($G_1 = 0.76$; $P = 0.38$).

Regardless, the mass of triploid workers was significantly greater than that of diploid workers. This result is consistent with previous studies in fire ants that have demonstrated an association between *S. invicta* ploidy and mass in workers (Goodisman et al. 1999; Krieger et al. 1999). It is unknown if variation in ploidy among workers affects worker function, as seems to be the case with triploid gynes, which ultimately fail to become reproductively active (Krieger et al. 1999). However, the variation in mass associated with ploidy would give triploid workers a survival advantage in cold climates. Thus environmental selection could favor triploid workers under certain conditions.

Finally, we discovered a significant effect of *Gp-9* genotype on worker mass in our polygyne population when rank-of-mass was considered as the response variable. Specifically, the mean rank-of-mass for *BB* workers was significantly lower than that for *Bb* or *bb* workers. This result was surprising for two reasons. First, the mean raw mass of *BB* workers was actually *higher* than the mean raw mass of *bb* workers, even though the mean rank-of-mass for *BB* workers was *lower* than for *bb* workers (Table 4). This paradoxical result arises from the fact that the variance in raw mass of *BB* workers was much higher than for the other genotypes. Thus the mean raw mass of *BB* workers was greater than of *bb* workers because there were many *BB* workers that were particularly large. But the median raw mass of *BB* workers was considerably lower than the median raw mass of *bb* workers.

The second reason why the relationship between *Gp-9* genotype and worker mass was unexpected was that it stands in contrast to previous findings for polygyne *S. invicta* gynes and workers. Earlier studies have consistently found that the mass of individuals varied with *Gp-9* genotype, but that the relationship was *BB* > *Bb* > *bb* (Ross and Keller 1998; Goodisman et al. 1999; Keller and Ross 1999; DeHeer 2002). Nevertheless, our results in this study can be explained by the fact that the previous study investigating the relationship between *Gp-9* genotype and worker mass sampled workers nonrandomly (Goodisman et al. 1999). In the earlier investigation, the genotypes of workers displaying a wide range of sizes were deliberately assayed, thereby resulting in a sample of workers whose mass was relatively high compared the true mass of workers found in polygyne nests. This differs from this study, where workers were randomly sampled. Consequently, if large workers were preferentially sampled in this previous study, and largest workers tended to be genotype *BB* because of the high variance in the mass of *BB* individuals, then *BB* workers could be found to be significantly more massive than workers of the other genotypes.

In conclusion, the variation in worker size associated with nest, sampling date, ploidy, and *Gp-9* genotype could be of substantial importance in *S. invicta*. Traits such as worker survivorship are related to worker mass, with larger workers surviving cold temperatures better than smaller workers (Calabi and Porter 1989). Thus heterozygous *Bb* workers may survive the winter with higher probability than *BB* or *bb* workers, because of their larger size. Worker genotype frequencies at *Gp-9* may also vary across the US

if natural selection operates on worker size (and by extension worker genotype) through differential viability related to annual temperature patterns. Moreover, ant workers of different size frequently undertake distinct tasks (Oster and Wilson 1978; Hölldobler and Wilson 1990) as is the case in *S. invicta* (Mirenda and Vinson 1981; Porter and Tschinkel 1986). Consequently, *BB* workers may engage in a greater variety of tasks than *Bb* or *bb* workers, because of the high variance in size that *BB* workers display. Finally, invasive ants tend to be relatively small compared to their noninvasive congeners. The explanation for this last trend remains unclear but may be related to selection for large colony size, polygyny, or particular competitive abilities (McGlynn 1999). Consequently, worker size in invasive ants is likely subject to a variety of biotic and abiotic selective forces, which alter the distribution of workers to match the environment over space and time.

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