Effects of a Single Gene on Worker and Male Body Mass in the Fire Ant *Solenopsis invicta* (Hymenoptera: Formicidae)

MICHAEL A. D. GOODISMAN,[1] PAUL D. MACK,[1] DEVON E. PEARSE,[1] AND KENNETH G. ROSS[2]

Department of Entomology, University of Georgia, Athens GA 30602–2603

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ABSTRACT This study examines the effects of *general-protein-9* (*Gp-9*) genotype on the body mass of polygyne (multiple-queens per nest) *Solenopsis invicta* Buren workers and males. We found that *Gp-9* genotype was significantly associated with variation in worker mass in field but not laboratory colonies. Moreover, triploid workers with 2 distinguishable genotypes ($Gp\text{-}9^{BBb}$ and $Gp\text{-}9^{Bbb}$) weighed significantly more than diploid workers with the heterozygous genotype ($Gp\text{-}9^{Bb}$). Our results, combined with those obtained from previous studies, indicate that *Gp-9* genotype, ploidy, social form, and colony queen number affect mass of *S. invicta* workers. We also discovered that *Gp-9* genotype significantly influenced the mass of haploid males reared in both field and laboratory environments. As a group, polygyne males were significantly lighter than monogyne males, even when *Gp-9* genotype was taken into account, indicating that social environment interacts with *Gp-9* genotype to influence male mass. Given that diploid males previously have been shown to be lighter than haploid males, 3 factors (*Gp-9* genotype, social form, and ploidy) are now known to affect the mass of male fire ants.

KEY WORDS *Solenopsis invicta*, fire ants, polygyny, caste, introduced species, genetic effects

INTRASPECIFIC VARIATION IN size fundamentally influences the behavior and fitness of all 3 castes (queens, males, and workers) of the eusocial Hymenoptera (Oster and Wilson 1978, Waddington 1988, Hölldobler and Wilson 1990, Ross and Matthews 1991, Schmid-Hempel 1992, Bourke and Franks 1995, Heinze and Tsuji 1995). For example, worker size is often associated with preferential performance of particular tasks (Hölldobler and Wilson 1990), whereas variation in the size of sexuals frequently is related to alternate reproductive strategies adopted by these individuals (Heinze and Tsuji 1995). The proximate factors promoting individual size or other morphological differences within castes have often remained difficult to establish, although such variation generally is attributed to environmental factors such as trophic differences during larval development (Hölldobler and Wilson 1990, Wheeler 1991, O'Donnell 1998, cf., Heinze and Buschinger 1989). Therefore, studies in the introduced fire ant *Solenopsis invicta* Buren that indicate that genotype at a single protein-encoding locus substantially influences variation in queen size are of considerable interest.

Previous investigations reported that the mass of queens originating from polygyne (multiple-queen) colonies was strongly associated with genotype at the locus *phosphoglucomutase-3* (*Pgm-3*) (Keller and Ross 1993b, 1995). However, recent results demonstrate that all phenotypic effects of genotype on queen mass are associated with the linked locus *general protein-9* (*Gp-9*) (Keller and Ross 1999). Introduced polygyne populations display 2 alleles at this locus, $Gp\text{-}9^{B}$ and $Gp\text{-}9^{b}$. Queens of genotype $Gp\text{-}9^{BB}$ are significantly more massive than those of genotype $Gp\text{-}9^{Bb}$ which, in turn, are significantly more massive than queens of genotype $Gp\text{-}9^{bb}$ (DeHeer et al. 1999, Keller and Ross 1999).

Polygyne workers and males also show genetic variation at the locus *Gp-9* (Ross 1997). Therefore, the possibility exists that *Gp-9* genotype influences mass in these 2 castes, as well as in queens. The primary purpose of this study was to test for such effects in polygyne *S. invicta* workers and males reared in both laboratory and field colonies. We expect that if workers of differing genotype vary in mass, they should follow the same mass hierarchy observed in queens ($Gp\text{-}9^{BB} > Gp\text{-}9^{Bb} > Gp\text{-}9^{bb}$). Similarly, we expect haploid $Gp\text{-}9^{B}$ males to be larger than $Gp\text{-}9^{b}$ males.

A secondary goal of this study is to investigate possible supplemental effects of ploidy on mass in the worker caste. Recent studies have demonstrated that a small percentage (≈12%) of workers in introduced *S. invicta* populations are triploid (Krieger et al. 1999). It has been shown earlier that *S. invicta* males that are diploid weigh significantly more than haploid males (Ross and Fletcher 1985), and we wanted to determine if ploidy level had similar effects on mass in the worker caste.

[1] Department of Genetics, University of Georgia, Athens GA 30602–7223.

[2] To whom correspondence should be addressed.

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Materials and Methods

Samples of Workers. Colonies headed by a single polygyne queen were established in the laboratory to examine variation in worker mass under uniform conditions (e.g., Ross 1997). Mated queens obtained from natural polygyne colonies were isolated in rearing chambers with worker adults and brood from their nests. These artificial single-queen colonies were provided with food and water for 8 wk, after which all pupae in the colonies were produced by the resident queen (O'Neal and Markin 1975). The queen and worker pupae were moved to a larger container and given excess food and water. After several additional months, we collected workers from 13 of these laboratory-reared colonies. Because all reproductive polygyne queens in introduced populations are heterozygous at the locus *Gp-9* (Ross 1997) and mate with a single haploid male, workers within any single laboratory colony necessarily possess either genotypes $Gp\text{-}9^{BB}$ and $Gp\text{-}9^{Bb}$ or genotypes $Gp\text{-}9^{Bb}$ and $Gp\text{-}9^{bb}$. In addition to the laboratory samples, we also collected workers from 101 polygyne colonies in the field. To obtain these samples, we disturbed the tops of nests, and used forceps and aspirators to capture the workers as they swarmed to the surface.

We assayed ≈47 workers (46.92 ± 0.92; mean ± SEM) from each of the 13 laboratory colonies and ≈16 workers (16.00 ± 0.02) from each of the 101 field colonies. A wide range of worker sizes was sampled to maximize the probability of detecting genotypic effects on worker mass. Each worker was weighed to the nearest hundredth of a milligram, and its genotype was then assayed at *Gp-9* using standard starch gel electrophoresis (DeHeer et al. 1999).

In addition to scoring the genotype of each worker at *Gp-9*, we also determined the ploidy of field-collected workers that had at least 1 copy of each *Gp-9* allele (workers collected from laboratory colonies apparently were all diploid, unpublished data). We can detect a proportion of triploid workers by examining the intensity of the *Gp-9* staining bands on the starch gels (Krieger et al. 1999). The 2 allelic bands of heterozygous diploid ($Gp\text{-}9^{Bb}$) workers appear equally intense. However, the staining patterns of triploid workers with the genotypes $Gp\text{-}9^{BBb}$ and $Gp\text{-}9^{Bbb}$ exhibit asymmetries in staining intensity of the 2 bands. Although triploid workers bearing 3 copies of the same allele probably exist (i.e., $Gp\text{-}9^{BBB}$ and $Gp\text{-}9^{bbb}$), they cannot be distinguished from diploid homozygotes using this technique.

Samples of Males. We collected mature adult males from polygyne laboratory and field colonies. Laboratory samples were obtained from 1 of the colonies from which workers were collected (see above); in total, 192 males were sampled randomly from this single colony. Field-sampled polygyne males were collected from the tops of 9 nests (16.33 ± 3.90 males per nest) as they initiated their mating flights. For comparison with the polygyne data, we also sampled 127 monogyne males from colonies in the field; only a single male was collected from each monogyne colony. All males were weighed to the nearest tenth of a milligram.

The *Gp-9* genotype of males cannot be assayed directly, because the product of this locus is not present in males (Ross 1997). Nevertheless, we can obtain the genotype of males at the closely linked, diallelic locus, *Pgm-3*. In introduced polygyne populations, the allele $Pgm\text{-}3^{A}$ is always associated with the allele $Gp\text{-}9^{B}$, whereas the alternate allele, $Pgm\text{-}3^{a}$, occurs in association with both *Gp-9* alleles (Ross 1997). Genotype at the locus *Pgm-3* thus provides substantial information concerning genotype at *Gp-9* in polygyne populations. Therefore, we assayed the genotype of all field- and laboratory-collected males at *Pgm-3* (methods in Shoemaker et al. 1992). The genotypes of polygyne males collected from the field also were determined at 4 other allozyme loci, *Aat-2*, *Est-4*, *G3pdh-1*, and *Pgm-1*, using starch gel electrophoresis. All 4 of these loci are diallelic and are unlinked to each other and to *Pgm-3* (Shoemaker et al. 1992).

Many males produced in introduced polygyne *S. invicta* populations are diploid (all monogyne males are haploid) (Ross and Fletcher 1985). We wanted to differentiate these diploid males from the haploid males in our analyses. Diploid males can be distinguished from haploid males genetically because the former usually display heterozygous patterns for the staining bands at 1 or more allozyme loci, whereas the latter never exhibit more than a single band at any locus. We used this information as a 1st step in differentiating the 2 types of males in our field samples (no diploid males were present in the laboratory colony).

The genetic method of determining ploidy will fail to detect diploid males that are homozygous at all assayed loci. To further refine our estimate of ploidy in polygyne males, we make use of the fact that diploid males are significantly heavier than haploid males (Ross and Fletcher 1985). We thus used the minimal mass of the genetically determined diploid males as a maximal cut-off mass for the putative haploid males. All genetically haploid males (single staining band at all loci) whose mass fell above this cut-off were excluded from analysis. Adjusting our sample in this manner is conservative from the standpoint of the haploid male data set, because we are reducing our sample size and potentially removing the largest class of haploid males (thereby compressing the range of masses for which a genotypic effect could be discerned). However, we note that eliminating these individuals from our analyses may lead to an underestimate of the mean mass of polygyne haploid males.

Statistical Tests. *Gp-9* has been shown to be under selection in polygyne populations (Ross 1997). To detect selection in this study, we tested the genotype frequencies in our worker and male samples for deviations from expected ratios via *G*-tests for goodness-of-fit. We used nonparametric methods to detect the effects of genotype on mass, because the mass values were not normally distributed. The Kruskal–Wallis test was applied when individuals were collected from a single colony (polygyne laboratory-sampled males),

Table 1. Mean mass (±SEM mg) of polygyne workers of differing *Gp-9* genotypes collected from laboratory and field colonies

Genotype	Laboratory (498)	Field (1,509)	All samples (2,007)
$Gp\text{-}9^{BB}$	1.65 ± 0.05 (238)	1.73 ± 0.04 (372)	1.70 ± 0.04 (610)
$Gp\text{-}9^{Bb}$	1.57 ± 0.05 (243)	1.54 ± 0.05 (1,090)	1.55 ± 0.02 (1,333)
$Gp\text{-}9^{bb}$	1.35 ± 0.18 (17)	1.20 ± 0.12 (47)	1.24 ± 0.07 (64)

Sample sizes are given in parentheses.

or when only a single ant per colony was obtained (monogyne males) (Sokal and Rohlf 1995). In other cases, we used the Scheirer–Ray–Hare extension of the Kruskal–Wallis test to correct for colony effects on genotype (Sokal and Rohlf 1995).

Results

Workers. *Laboratory Colonies.* Examination of worker genotypes from the laboratory colonies revealed that 12 colonies were headed by queens mated to a $Gp\text{-}9^{B}$ male, and 1 colony was headed by a queen mated to a $Gp\text{-}9^{b}$ male. Therefore, workers in the former 12 colonies were of genotypes $Gp\text{-}9^{BB}$ and $Gp\text{-}9^{Bb}$, and workers in the latter colony were of genotypes $Gp\text{-}9^{Bb}$ and $Gp\text{-}9^{bb}$.

We first tested the distribution of the 2 worker genotypes within each colony for deviations from the 1:1 ratio expected under Mendelian segregation. Only a single colony showed significant deviations from the expected ratio (*G*-tests within each colony), and this deviation is not significant when we use sequential Bonferroni corrections to account for the multiple tests performed (Rice 1989). We next pooled the genotypes across the 12 colonies with workers of genotypes $Gp\text{-}9^{BB}$ and $Gp\text{-}9^{Bb}$ to detect any slight systematic biases in segregation ratios. However, we found no evidence for any such bias even in this larger sample ($G = 1.17$, df = 1, $P > 0.25$). We thus conclude that the *Gp-9* genotypic ratios of workers in these laboratory colonies do not deviate from Mendelian expectations.

The mean masses of laboratory-reared workers of each genotype are given in Table 1, and the distributions of masses are displayed in Fig. 1A. We note that the mean and median masses are in the predicted order, with the order of worker mass $Gp\text{-}9^{BB} > Gp\text{-}9^{Bb} > Gp\text{-}9^{bb}$. However, significant components of the variation in mass in these laboratory samples cannot be explained by either colony or genotype (colony, $H = 10.50$, df = 12, $P > 0.5$; genotype, $H = 0.83$, df = 2, $P > 0.6$; interaction, $H = 14.77$, df = 11, $P > 0.1$). Therefore, the laboratory data cannot reject the null hypothesis of no association between worker mass and genotype at the locus *Gp-9*.

Field Colonies. Although the *Gp-9* genotypes of the males that fathered workers in the field remain unknown, it is still possible to conduct simple tests for deviations from expected segregation ratios. Because

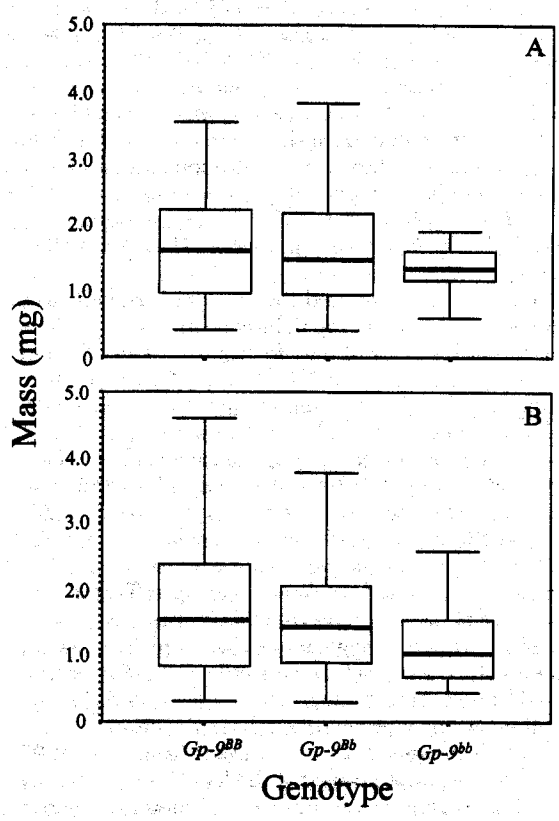

Fig. 1. Distributions of masses of polygyne workers of differing *Gp-9* genotypes collected from (A) laboratory and (B) field colonies. Each box displays the interquartile range and median worker mass, and the whiskers mark the minimal and maximal values.

all polygyne queens are $Gp\text{-}9^{Bb}$, 50% of polygyne workers should be heterozygous (the ratio of the remaining 50% of homozygous $Gp\text{-}9^{BB}$ or $Gp\text{-}9^{bb}$ workers depends on the genotypes of the fathers). Therefore, we may pool the 2 homozygous classes and test for deviations from the expected 1:1 heterozygote: homozygote ratio. The observed proportions of heterozygous and homozygous genotypes deviate significantly from this expectation ($G = 309.07$, df = 1, $P < 0.0001$), because of the substantially greater number of heterozygous than homozygous workers observed.

We next turn to the masses of the field-collected workers. Table 1 gives the mean masses and Fig. 1B displays the distribution of masses for these individuals. As in the laboratory colonies, the mean and median masses are in the predicted order ($Gp\text{-}9^{BB} > Gp\text{-}9^{Bb} > Gp\text{-}9^{bb}$). Also, the mean mass for each genotype does not differ significantly between the laboratory- and field-collected workers ($Gp\text{-}9^{BB}$, $t = 1.25$, df = 505, $P > 0.2$; $Gp\text{-}9^{Bb}$, $t = 0.42$, df = 792, $P > 0.6$; $Gp\text{-}9^{bb}$, $t = 0.69$, df = 31, $P > 0.4$), although the means for the $Gp\text{-}9^{BB}$ and $Gp\text{-}9^{bb}$ workers are more extreme in the field samples. However, in contrast to the results obtained from the laboratory colonies, we find that both colony and genotype significantly affect variation

in worker mass (colony, $H = 177.37$, df = 100, $P < 0.0001$; genotype, $H = 13.53$, df = 2, $P < 0.005$; interaction, $H = 121.05$, df = 131, $P > 0.7$). Therefore, *Gp-9* genotype is associated with variation in worker mass in field colonies. Not surprisingly, when we combined all the worker data from the laboratory and the field (means given in Table 1), we also found a significant effect of colony and genotype on worker mass (colony, $H = 191.42$, df = 113, $P < 0.0001$; genotype, $H = 14.50$, df = 2, $P < 0.001$; interaction, $H = 139.06$, df = 144, $P > 0.6$).

Finally, we considered the effects of triploidy and genotype on mass in the field-collected workers. The mean masses (±SEM mg) for $Gp\text{-}9^{BBb}$, $Gp\text{-}9^{Bbb}$, and $Gp\text{-}9^{Bb}$ workers are 2.36 ± 0.17, 2.15 ± 0.10, and 1.49 ± 0.02, respectively. We found that both colony and ploidy significantly influence variation in mass of workers bearing at least 1 copy of each allele (colony, $H = 136.91$, df = 100, $P < 0.01$; ploidy, $H = 43.62$, df = 2, $P < 0.0001$; interaction, $H = 30.41$, df = 54, $P > 0.9$).

The effect of triploidy on mass could confound the effect of genotype on mass in our worker samples. For instance, if triploid workers make up different proportions of the 3 putative diploid *Gp-9* genotypes, and triploid workers weigh more than diploid workers, then all the variation that we ascribe to *Gp-9* genotype could result from triploidy alone. We tested this hypothesis using a previously collected worker data set in which the genotype of 1 worker from each of 97 field colonies was assayed at the loci *Gp-9* and *Pgm-1*. Triploid workers can be detected by their asymmetric staining patterns at the locus *Pgm-1* (see above). Moreover, because *Pgm-1* is unlinked to *Gp-9*, we can test the hypothesis that triploidy (as determined by staining pattern at *Pgm-1*) is randomly distributed among the 3 *Gp-9* phenotypic classes. In total, 62, 33, and 2 workers were of putative genotypes $Gp\text{-}9^{BB}$, $Gp\text{-}9^{Bb}$, and $Gp\text{-}9^{bb}$, respectively, and 4, 5, and 0 triploid workers were detected using *Pgm-1* in these 3 classes. The observed distribution of triploid workers does not differ significantly from that expected under the null hypothesis of no association between ploidy and *Gp-9* allele composition ($G = 2.00$, df = 2, $P > 0.3$). Consequently, we have no evidence that workers with certain *Gp-9* staining patterns are more likely to be triploids. Therefore, our conclusion that both *Gp-9* genotype and ploidy affect mass of field-collected workers appears valid.

Males. *Laboratory Colony.* Genetic analysis of female progeny from the single laboratory colony confirmed that the two-locus genotypes of the single mother queen and her male mate were $Pgm\text{-}3^{Aa}/Gp\text{-}9^{Bb}$ and $Pgm\text{-}3^{A}/Gp\text{-}9^{B}$, respectively. Because the $Pgm\text{-}3^{A}/Gp\text{-}9^{b}$ haplotype is absent in this study population (Ross 1997), the mother was necessarily a *cis* heterozygote. The genotypes of a sample of males were assayed at several microsatellite loci and revealed that some males in this colony were produced by unmated daughters of the mother queen (J. Gadau, unpublished data). Nevertheless, $Pgm\text{-}3^{A}$ and $Pgm\text{-}3^{a}$ males in this colony necessarily possess the genotypes $Gp\text{-}9^{B}$ and $Gp\text{-}9^{b}$, respectively (the *Gp-9* genotypes being of direct interest to this study), regardless of whether they were produced by the mother queen or her daughters.

Table 2. Mean mass (±SEM mg) of polygyne and monogyne haploid males of differing *Pgm-3* genotypes collected from laboratory and field colonies

Genotype	Polygyne			Monogyne
	Laboratory (192)	Field (22)	All samples (214)	Field (127)
$Pgm\text{-}3^{A}$	7.00 ± 0.06 (83)	7.05 ± 0.28 (4)	7.00 ± 0.05 (87)	8.33 ± 0.08 (85)
$Pgm\text{-}3^{a}$	5.86 ± 0.04 (109)	5.94 ± 0.15 (18)	5.88 ± 0.04 (127)	8.32 ± 0.11 (42)

In polygyne populations, *Pgm-3* genotype is predictive of *Gp-9* genotype, which is the focus of this study. Sample sizes are given in parentheses.

We first examined the patterns of allelic segregation in the 192 males. A total of 83 $Pgm\text{-}3^{A}$ and 109 $Pgm\text{-}3^{a}$ males was sampled (Table 2). This distribution showed a marginally significant deviation from the 1:1 ratio expected if all males were produced by the single mother queen ($G = 3.53$, df = 1, $0.05 < P < 0.10$). This result is particularly notable because male production by unmated daughters of the mother queen in this colony would be expected to increase the frequency of $Pgm\text{-}3^{A}$ males, yet our sample showed a slight deficit of males possessing this allele.

We next turned to the means (Table 2) and distributions (Fig. 2A) of masses for these laboratory males. The means of the 2 *Pgm-3* genotypic classes differ significantly from one another ($H = 117.08$, df = 1, $P < 0.0001$). Given the complete association between *Pgm-3* and *Gp-9* alleles in males of this colony, our laboratory data strongly suggest an effect of *Gp-9* genotype on mass in haploid males, with $Gp\text{-}9^{B}$ males heavier than $Gp\text{-}9^{b}$ males.

Polygyne Field Colonies. Diploid males (as determined genetically) constituted 78.2% of the field samples, and the minimal diploid male mass was 7.8 mg. All genetically haploid males whose mass fell above this value were disregarded in the haploid male analysis. The means for the 2 *Pgm-3* genotypes (which are predictive of *Gp-9* genotype) in field-sampled, haploid, polygyne males, given in Table 2, do not differ significantly from those of males of the same genotype sampled from the laboratory colony ($Pgm\text{-}3^{A}$, $t = 0.17$, df = 3, $P > 0.8$; $Pgm\text{-}3^{a}$, $t = 0.52$, df = 19, $P > 0.6$) (distributions of masses displayed in Fig. 2B). More importantly, field-collected $Pgm\text{-}3^{A}$ males weighed significantly more than $Pgm\text{-}3^{a}$ males (colony, $H = 2.77$, df = 5, $P > 0.7$; genotype, $H = 6.09$, df = 1, $P < 0.05$; interaction, $H = 1.36$, df = 2, $P > 0.5$). When we combined the laboratory and field samples (means given in Table 2), we also observed a highly significant effect of *Pgm-3* genotype on haploid male mass (colony, $H = 3.31$, df = 6, $P > 0.5$; genotype, $H = 125.17$, df = 1, $P < 0.0001$; interaction, $H = 1.56$, df = 3, $P > 0.5$).

We next examined the distribution of masses for the diploid males of differing *Pgm-3* genotype (the surro-

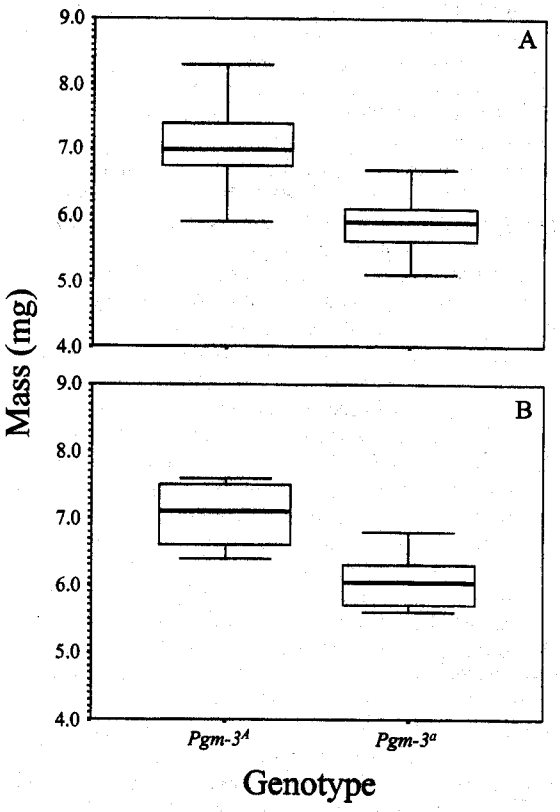

Fig. 2. Distributions of masses of haploid polygyne males of differing *Pgm-3* genotypes collected from (A) laboratory and (B) field colonies. In polygyne populations, *Pgm-3* genotype is predictive of *Gp-9* genotype, which is the focus of this study. Each box displays the interquartile range and median male mass, and the whiskers mark the minimal and maximal values.

gate for *Gp-9* genotype). We found that the mean masses of $Pgm\text{-}3^{AA}$, $Pgm\text{-}3^{Aa}$, and $Pgm\text{-}3^{aa}$ genetically diploid males are 9.00 ± 0.20, 9.39 ± 0.06, and 9.54 ± 0.19 mg, respectively. The order of these means runs counter to that predicted from the haploid male and diploid female data (predicted order of masses: $Pgm\text{-}3^{AA} > Pgm\text{-}3^{Aa} > Pgm\text{-}3^{aa}$), and no significant differences in mass exist among the 3 genotypes (colony, $H = 26.91$, df = 8, $P < 0.001$; genotype, $H = 0.99$, df = 2, $P > 0.6$; interaction, $H = 4.60$, df = 4, $P > 0.3$). Therefore, in contrast to the results obtained for haploid males, genotype at the locus *Pgm-3* is not a strong predictor of mass in diploid males.

Monogyne Field Colonies. Table 2 gives the mean masses of $Pgm\text{-}3^{A}$ and $Pgm\text{-}3^{a}$ monogyne males. The mean masses of monogyne males of the 2 genotypes do not differ significantly ($H = 0.13$, df = 1, $P > 0.7$). This result is consistent with expectations. All monogyne populations are fixed for allele $Gp\text{-}9^{B}$, and therefore, in contrast to polygyne populations, *Pgm-3* genotype in monogyne populations does not provide information concerning variation at *Gp-9*. We next compared the mean mass of all monogyne males to that of all polygyne males and found that the males of the 2 social forms differ significantly in mass ($H = 215.90$, df = 1, $P < 0.0001$). Finally, we wanted to determine if social form had an effect on mass independent of *Pgm-3* (*Gp-9*) genotype. We thus compared the masses of polygyne $Pgm\text{-}3^{A}$ males (all of which are $Gp\text{-}9^{B}$) to all monogyne males (all of which also are $Gp\text{-}9^{B}$). Once again, we found that polygyne $Pgm\text{-}3^{A}$ males weigh significantly less than monogyne males ($H = 120.67$, df = 1, $P < 0.0001$), indicating a strong effect of social form on haploid male mass after accounting for genotype.

Discussion

We have demonstrated that genotype at a single locus, *Gp-9*, is associated with variation in the mass of polygyne workers and males of the introduced fire ant *S. invicta*. Previous studies established that this locus influences mass in queens (Keller and Ross 1993a, 1999; Ross and Keller 1998; DeHeer et al. 1999). Therefore, a single genetic locus influences variation in mass in all 3 castes of the polygyne social form of this ant.

Workers. A significant effect of *Gp-9* genotype on mass was detected in field samples, although no such effect was detected in laboratory samples. There are at least 2 nonexclusive explanations for why we failed to detect an effect in the latter case. The 1st possibility is that workers reared in laboratory colonies fail to display the full range of variation possible given their genotype, because the laboratory environment somehow buffers against *Gp-9* effects. Consistent with this explanation is the fact that the mean mass of $Gp\text{-}9^{BB}$ workers assayed from field colonies was greater than that of $Gp\text{-}9^{BB}$ workers assayed from laboratory colonies, and field-reared $Gp\text{-}9^{bb}$ workers weighed less than laboratory-reared $Gp\text{-}9^{bb}$ workers. Moreover, laboratory workers exhibit the genotypic ratios expected if selection does not act on *Gp-9* in this context, whereas the genotype distribution of the field-sampled workers deviates significantly from expected distributions (Ross 1997). This pattern is expected if the effects of *Gp-9* genotype penetrate incompletely under laboratory conditions.

The 2nd, simpler explanation proposes that the mean masses of laboratory-reared workers of different *Gp-9* genotypes were not significantly different because our test lacked power. That the mean mass of each genotype did not differ significantly in the laboratory and field samples strongly supports this 2nd hypothesis. Moreover, the significance of the differences in mean masses for the 3 *Gp-9* genotypes is greater if data from both the laboratory and field colonies are combined than if the field colonies are considered alone. This result suggests that if more laboratory-reared workers had been sampled, a significant effect of *Gp-9* genotype on mass would have been detected.

For at least 2 reasons, neither of our samples is likely to yield accurate point estimates for the mean mass of workers of differing *Gp-9* genotypes in natural polygyne populations. First, workers were sampled non-

randomly from nests; workers randomly sampled from polygyne colonies weigh less, on average, than the samples obtained in this study (Goodisman and Ross 1996). Second, a nonrandom proportion of workers could not be scored at *Gp-9* (17.8% of the laboratory workers and 6.6% of the field workers failed to display staining bands of sufficient intensity to score). The mean mass for these missing laboratory and field workers was 0.89 and 0.71 mg, respectively, values substantially lower than the mean masses for all 3 *Gp-9* genotypes (Table 1).

The effect of *Gp-9* genotype on worker mass in introduced polygyne populations may, in part, explain why polygyne workers are significantly smaller than monogyne workers (Greenberg et al. 1985, Goodisman and Ross 1996), a phenomenon that, until now, has been ascribed to environmental effects associated with queen number (e.g., Goodisman and Ross 1996). Monogyne populations are fixed for the $Gp\text{-}9^B$ allele; therefore, all monogyne workers are of genotype $Gp\text{-}9^{BB}$ (Ross 1997). Our results indicate that a population of workers of genotype $Gp\text{-}9^{BB}$ (i.e., monogyne workers) would weigh more, on average, than a population composed of workers of all 3 genotypes (i.e., polygyne workers). Although other factors such as queen number, larval density, colony size, and colony age probably affect individual worker mass within colonies (Porter and Tschinkel 1985a, Tschinkel 1993, Goodisman and Ross 1996), genotype at the locus *Gp-9* may play an important role in generating the worker mass differences that distinguish the 2 social forms of *S. invicta*.

The variation in worker mass detected in this study may result from differences in exoskeleton size that are set during larval growth. The strong correlation between individual body weight and head width found in monogyne *S. invicta* workers bolsters this explanation (Porter and Tschinkel 1985b). Alternatively, the mass of polygyne workers of different *Gp-9* genotypes may vary because genotype at this locus influences mass accumulation after the workers eclose. Support for this hypothesis arises from a study of polygyne *S. invicta* queens, which found that variation in mass associated with *Pgm-3* (*Gp-9*) genotype resulted from differential fat accumulation during adult maturation, not from differences in exoskeleton size (Keller and Ross 1993b).

Effects of Ploidy on Worker Mass. In accord with our predictions, triploid and diploid workers differed significantly in mass independent of *Gp-9* genotype (see also Krieger et al. 1999). This result is consistent with the fact that diploid polygyne *S. invicta* males weigh significantly more than haploid males (Ross and Fletcher 1985, this study). The effect of ploidy on male mass may result from changes in cell volume associated with variation in chromosome number (Grosch 1945). Perhaps a similar explanation accounts for the increase in mass with increased ploidy in workers.

Our results suggest that the effects of ploidy override the effects of *Gp-9* genotype in workers. That is, workers of the 2 detectable triploid genotypes ($Gp\text{-}9^{BBb}$ and $Gp\text{-}9^{Bbb}$) do not differ significantly in mass, although they differ in *Gp-9* allelic composition, and workers of both genotypes are heavier than either $Gp\text{-}9^{BB}$ or $Gp\text{-}9^{Bb}$ diploid workers. Triploidy also has been detected in prereproductive winged queens (Krieger et al. 1999) and, in contrast to males and workers, queens with higher ploidy (triploids) are not uniformly heavier than queens of lower ploidy (diploids) (Keller and Ross 1999). Rather, the mass of a polygyne alate queen is determined by the number of $Gp\text{-}9^B$ alleles that she possesses.

Consequences of Variation in Worker Mass. The variation in worker mass associated with *Gp-9* genotype probably has far reaching consequences in polygyne *S. invicta* societies. For example, worker survivorship in *S. invicta* is related to worker size. Larger workers tend to live longer and survive cold conditions better than smaller workers (Markin and Dillier 1971, Porter and Tschinkel 1985b, Calabi and Porter 1989). Therefore, workers of different *Gp-9* genotypes probably differ in survivorship, the relative survivorship of the 3 genotypes being given by the hierarchy $Gp\text{-}9^{BB} > Gp\text{-}9^{Bb} > Gp\text{-}9^{bb}$.

Another important consequence of the interaction between *Gp-9* genotype and worker mass relates to the behavioral differences associated with worker size. Studies in other ants suggest that selective pressures in the environment, combined with the fact that workers of different sizes perform certain tasks more efficiently (size-based polyethism), may shape the distribution of worker size within colonies (Oster and Wilson 1978, Hölldobler and Wilson 1990, Schmid-Hempel 1992). Previous studies in *S. invicta* have found evidence for such size-based polyethism, with smaller workers tending to care for brood and larger workers specializing on foraging (Wilson 1978; Mirenda and Vinson 1981; Porter and Tschinkel 1985b, 1986). If the differences in worker mass observed in this study reflect differences in the external size of workers, then our results suggest that variation in *Gp-9* genotype may be an important factor in determining the structure of polyethism in *S. invicta* colonies.

Males. Our results demonstrate that genotype at the locus *Pgm-3*, which is tightly linked to *Gp-9*, significantly influences haploid male mass in introduced polygyne populations of *S. invicta*. Recent studies have shown that *Pgm-3* effects on mass in *S. invicta* queens actually are caused entirely by variation at the linked locus *Gp-9* (Keller and Ross 1999). We thus presume that the differences in male mass observed in this study are related to genotype at the locus *Gp-9* rather than *Pgm-3*, and hereafter we will refer to the genotype effect on mass as being correlated with *Gp-9* genotype.

In both laboratory and field colonies, polygyne $Gp\text{-}9^B$ males were significantly more massive than polygyne $Gp\text{-}9^b$ males, and, unlike the situation for workers, there is no indication that *Gp-9* genotype shows distinct patterns of expression in the 2 environments. Moreover, the male samples probably reflect the range of variation observed in natural populations, because all specimens could be scored readily at *Pgm-3*.

In contrast to the results obtained for haploid polygyne males, we failed to find any significant relationship between *Pgm-3* genotype and mass in monogyne males. This result was expected because monogyne males of both *Pgm-3* genotypes presumably are of genotype $Gp\text{-}9^B$ (Ross 1997). The genetic differences between monogyne and polygyne males at *Gp-9* may account to some extent for the observation that monogyne males are larger than polygyne males. However, the presence of the $Gp\text{-}9^b$ allele in the polygyne population cannot explain all of the variation between monogyne and polygyne males, because we found that $Gp\text{-}9^B$ males originating from polygyne colonies were significantly lighter than monogyne males. Therefore, social form appears to have an effect on male mass independent of *Gp-9* genotype. This result is in accord with previous studies of *S. invicta* that found that social form also influenced variation in queen mass independent of genotype at *Gp-9* (Keller and Ross 1993b, 1995; DeHeer et al. 1999).

We suggest that the variation in male mass associated with *Gp-9* genotype detected in this study probably results from differences in exoskeleton size. The dry mass of monogyne *S. invicta* males increases by only 6% after eclosion (Tschinkel 1993). This growth probably cannot account for the large differences in mass associated with *Gp-9* genotype in polygyne colonies.

Effect of Gp-9 *Genotype on Diploid Male Mass.* *Gp-9* genotype was not significantly associated with variation in diploid male mass. It is possible that we failed to detect a trend because the effect of *Gp-9* genotype is suppressed in these males. However, we suspect that the negative result is, at least in part, related to the fact that *Pgm-3* genotype (our surrogate for *Gp-9* genotype) is not perfectly correlated with *Gp-9* genotype (Ross 1997). In this polygyne population, the $Pgm\text{-}3^A$ allele is always linked to the $Gp\text{-}9^B$ allele. However, the $Pgm\text{-}3^a$ allele is associated with both the $Gp\text{-}9^B$ and $Gp\text{-}9^b$ alleles. Thus, the lack of a perfect association between the 2 markers may have obscured any *Gp-9* effect on mass in our diploid male samples. In fact, we expect the imperfect relationship between *Pgm-3* and *Gp-9* genotype to confuse the mass-genotype relationship in diploid males more than in haploid males, because a greater frequency of diploid males possess the potentially confounding $Pgm\text{-}3^a$ allele.

Consequences of Variation in Male Mass. The distribution of genotype $Pgm\text{-}3^A$ ($Gp\text{-}9^B$) and $Pgm\text{-}3^a$ ($Gp\text{-}9^b$) males collected from our laboratory colony showed a marginally significant deviation from the expected 1:1 ratio, there being fewer $Pgm\text{-}3^A$ ($Gp\text{-}9^B$) males present in the sample than expected (Table 2). (Because of the incomplete information carried by *Pgm-3* concerning *Gp-9* genotype, it is not possible to conduct a test for such deviations in the field samples.) A previous study of polygyne field-collected males in this population found a slightly lower frequency of the $Pgm\text{-}3^A$ allele in males than in their mothers, a result that also suggests a deficit of $Gp\text{-}9^B$ males in the wild (Ross 1992). Thus, some evidence exists for the presence of weak endogenous selection acting on polygyne haploid males of differing *Gp-9* genotypes. However, it is unclear if any such selection is directly related to male weight, because no studies have examined the relationship between male viability and male mass.

An alternative explanation for the slight deviations in male allele frequencies is that polygyne workers selectively execute $Gp\text{-}9^B$ males. Such behavior would be consistent with previous studies in introduced *S. invicta*, which have shown that polygyne workers preferentially execute $Gp\text{-}9^{BB}$ queens as they mature (Keller and Ross 1993a, 1998). It is possible that the corresponding $Gp\text{-}9^B$ males also are subject to aggression by nestmate workers.

Regardless of whether differences in *Gp-9* genotype influence male viability, many males of both genotypes survive long enough to participate in mating flights, compete for mates, and successfully inseminate queens (Ross 1997). Differences in male mass associated with *Gp-9* genotype may translate into differential reproductive strategies at this stage. Indeed, several studies of ant mating behavior have found that larger males enjoy more mating opportunities or partake in different mating behavior than smaller males (see references in Hölldobler and Wilson 1990, Bourke and Franks 1995, Heinze and Tsuji 1995). Thus, *Gp-9* genotype may influence the reproductive success of polygyne fire ant males.

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