Behav Ecol Sociobiol (2002) 51:287–295
DOI 10.1007/s00265-001-0431-5

ORIGINAL ARTICLE

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Experimental conversion of colony social organization by manipulation of worker genotype composition in fire ants (*Solenopsis invicta*)

Received: 10 July 2001 / Revised: 19 October 2001 / Accepted: 23 October 2001 / Published online: 4 December 2001

Abstract Previous studies have shown that colony social organization in *Solenopsis invicta* is under strong genetic control. Colonies containing some proportion of workers with the *Bb* or *bb* genotypes at the gene *Gp-9* display polygyne social organization (multiple reproductive queens per colony), whereas colonies with only *BB* workers express monogyne organization (single reproductive queen per colony). The hypothesis that the presence of workers bearing the *b* allele confers the polygyne social phenotype on a colony leads to the prediction that social organization can be manipulated by experimentally altering frequencies of adult workers bearing this allele. We did this by replacing queens in colonies of each social form with single queens of the alternate form, which differ in *Gp-9* genotype. As worker *Gp-9* genotype compositions changed, experimental colonies switched to the alternate social organization. These switches occurred when frequencies of workers with the *b* allele passed an identifiable threshold, such that colonies with fewer than 5% such workers behaved like monogyne colonies and those with more than 10% behaved like polygyne colonies. Our data thus confirm the prediction that colony social organization in this ant can be altered by manipulating adult worker genotype compositions, and thereby support the hypothesis that the expression of polygyny requires the presence of adult workers bearing the *b* allele at *Gp-9*.

Keywords Behavioral genetics · Fire ants · Polygyny · Social organization · *Solenopsis invicta*

Communicated by J. Heinze

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Introduction

An important goal in biology is to understand the origin of complex social behavior, one of the principal transitions in the history of life (Maynard Smith and Szathmáry 1995; Keller and Reeve 1999). Most students of social evolution acknowledge that a full understanding of the origin and elaboration of sociality requires the construction of increasingly realistic models that incorporate details of the genetic architecture of fundamental social traits. That is, comprehensive selection models likely to significantly illuminate the processes of social evolution will not only take into account pertinent features of the life histories of appropriate social or transitional taxa, they will also incorporate information on the numbers of genes influencing the expression of key social traits, the relative magnitude of the effects of such genes, patterns of gene interaction (epistasis), and the norms of reaction of social phenotypes (see e.g., Grafen 1984; Crozier and Pamilo 1996; Robinson et al. 1997; Wolf et al. 1998; Robinson 1999).

Despite the acknowledged importance of determining the genetic components of complex social behavior, relatively few studies have succeeded in demonstrating that the expression of fundamental social traits has a heritable basis. As examples, caste or morph differentiation is proposed to be under genetic influence in some stingless bees and ants (Kerr 1974; Winter and Buschinger 1986; Fersch et al. 2000; Fraser et al. 2000), worker task performance can have a genetic component in honey bees, ants, and wasps (Snyder 1992; Hunt et al. 1995, 1998; O'Donnell 1996; Robinson et al. 1997), individual reproductive roles have a heritable component in honey bees (Moritz and Hillesheim 1985; Page and Robinson 1994; Moritz et al. 1996; Montague and Oldroyd 1998), and social organization of colonies can have a genetic influence in sweat bees and ants (Cahan et al. 1998; Ross and Keller 1998; Plateaux-Quénu et al. 2000). Among such studies, few have convincingly demonstrated a relatively simple genetic architecture featuring one or few genes of major effect (Moritz 1988; Hunt et al. 1995; Ross and

Keller 1998), and in only one case has a candidate gene of major effect been identified and fully characterized (Krieger and Ross, in press).

The latter case concerns the gene *Gp-9* in the fire ant *Solenopsis invicta*. This ant species exhibits two contrasting types of colony social organization, one in which colonies have a single reproductive (egg-laying) queen (characteristic of the *monogyne* social form) and one in which colonies have several to many reproductive queens (characteristic of the *polygyne* form) (Ross and Keller 1995). The gene *Gp-9* has two electrophoretically detectable alleles in introduced populations in the USA, the *B* allele, which is the only allele found in the monogyne form, and the *b* allele, which occurs together with the *B* allele in the polygyne form (Ross 1997). Effectively all reproductive queens in polygyne nests are *Bb* heterozygotes. Neither homozygous genotype is represented among these queens because polygyne workers destroy queens with the *BB* genotype before they become fully reproductively competent (Keller and Ross 1998) and queens with the *bb* genotype apparently die of intrinsic defects early in adult life (Ross 1997; DeHeer et al. 1999). Polygyne queens may mate with haploid males bearing either allele (male ants are usually haploid), so polygyne nests normally contain workers of all three genotypes. However, because adult workers with the *bb* genotype also suffer lethal defects, the vast majority of polygyne workers bearing the *b* allele are heterozygotes.

Because the $Gp\text{-}9^b$ allele is invariably represented among the workers and queens of polygyne *S. invicta* colonies, it appears to be an indispensable genetic component in the expression of the polygyne social organization. This idea is supported by extensive laboratory experiments that have revealed that colonies containing adult *Bb* workers behave like typical polygyne colonies by tolerating multiple *Bb* reproductive queens, whereas colonies with only *BB* workers behave like typical monogyne colonies by tolerating only a single *BB* queen (Ross and Keller 1998). It is important to recognize that the adult workers determine through their collective actions (aggression or tolerance) which queens, and how many, are allowed to survive and reproduce in a colony, so that the workers are the proximate agents controlling colony social organization (Fletcher and Blum 1983; Keller and Ross 1993, 1998). Thus, the presence of multiple heterozygous queens (defining polygyny) or of a single homozygous *BB* queen (defining monogyny) is the outcome of collective worker aggression or tolerance displayed toward individual queens attempting to become egg layers. The influence of worker *Gp-9* genotype composition on colony tolerance of queens appears to be very strong relative to the influence of non-genetic factors such as worker social experience, social form of origin of queens, or queen fecundity (Ross and Keller 1998); that is, the expressivity of *Gp-9* is virtually complete with respect to its effects on colony social behavior. The recent sequencing of *Gp-9* and identification of its product as an odorant-binding protein, a crucial molecular component of insect chemoreception, suggest that the gene product may be involved in the recognition and subsequent discrimination of queens by workers, which constitute the proximate behavioral processes by which social organization is regulated (Krieger and Ross, in press).

The results summarized above lead to a simple null hypothesis for the genetic regulation of colony social organization in *S. invicta*: colonies containing some minimal proportion of workers with the $Gp\text{-}9^b$ allele (heterozygotes, in general) will express the polygyne social organization, whereas colonies lacking such workers will express the monogyne social organization. A strong, testable prediction of this null hypothesis is that colony social organization can be changed by manipulating the frequencies of adult *Bb* workers in a colony. In this paper, we describe a study in which we conducted such manipulations by replacing the queens in colonies of each social form with queens of the alternate form. The resulting changes in social behavior in the test colonies corresponded in the predicted manner with the appearance or loss of *Bb* workers. Moreover, direct estimation of worker genotype frequencies at each assay point allowed us to infer the minimum proportion of *Bb* workers needed for the expression of polygyny, that is, the genotype frequency threshold for a transition in colony social organization.

Methods

Source colonies for experiments

Thirty-four monogyne *S. invicta* colonies containing their single reproductive queen were collected in Morgan, Oglethorpe, and Putnam Counties, Georgia, during late spring 2000. Also collected at this time in Oconee County, Georgia, were 22 polygyne *S. invicta* colonies with their multiple queens. All colonies were returned to the laboratory, separated from the soil, and maintained individually in laboratory-rearing units under standard conditions (Jouvenaz et al. 1977; Ross 1988). Each rearing unit consisted of a plastic tray (26 ×40×8 cm) containing two 14-cm-diameter plastic petri dishes with dark covers and moistened plaster bottoms that served as nests. All colonies and the assay fragments derived from them were fed daily on a diet of frozen crickets and pureed vegetables with sugar (Ross 1988).

Following establishment of the colonies in the laboratory, samples of 15 workers from each monogyne colony and 20–49 workers (mean=35.1) from each polygyne colony were subjected to horizontal starch gel electrophoresis to determine genotypes at *Gp-9* (see DeHeer et al. 1999 for methods). This genotypic information helped us to confirm the social form of each source colony as well as determine the baseline *Gp-9* genotype frequencies in the experimental units derived from polygyne colonies (all monogyne colonies contained only *BB* workers). The 95% confidence intervals around the genotype frequency estimates for polygyne colonies were obtained by employing a jackknife procedure to estimate the variances and then assuming the *t*-distribution (Weir 1996).

Adoption of queens of the alternate social form in experimental colonies

An essential task in this study was to replace the reproductive queen or queens of colonies of one social form with a queen of the alternate form, in order to cause a gradual changeover of adult workers with different *Gp-9* genotypes that would, in turn, be expected to produce a shift in colony social behavior. This task is difficult because colonies are very reluctant to accept queens of the al-

ternate form (Ross and Keller 1998). We succeeded in getting queens adopted into colonies of the alternate form by (1) dequeening host colony fragments containing several thousand worker adults and brood for a period of 5 days, (2) gradually giving brood to the adoptive queen and only later (over a period of several days) adding adult workers to the fragment, (3) frequently blowing human breath on the queen and workers massed around her, (4) repeatedly chilling the fragment, and (5) occasionally spraying the queen and workers massed around her with water mist. Fifteen colony fragments of each form were used as recipient colonies for adoptive queens of the alternate form. Each polygyne fragment was derived from a separate polygyne source colony, whereas three of the monogyne fragments were derived from monogyne source colonies used previously as sources of monogyne fragments (that is, only 12 of the 15 fragments originated from separate source colonies and can be considered as wholly independent replicates). All 15 monogyne worker fragments given polygyne queens (PQMW treatment) accepted the queens and developed normally over the course of the study. Only 11 of the polygyne worker fragments given monogyne queens (MQPW treatment) did so and are represented in the results presented below.

Colony assays

The purpose of these assays was to ascertain whether experimental colonies behaved as typical monogyne or polygyne colonies at different points over the course of the experiment. The basic assay involved introducing supernumerary reproductive polygyne queens (bearing the *Bb* genotype) into queenright or queenless test colonies and determining whether the introduced queens were accepted by the resident workers; monogyne colonies normally destroy *Bb* queens, whereas polygyne colonies typically accept several such queens (see Ross and Keller 1998). For the PQMW treatment in this study, the assay involved introducing a single polygyne (*Bb*) queen into each queenright test colony and determining if the introduced queen survived for 24 h along with the resident *Bb* queen. For the MQPW treatment, the assay involved introducing three polygyne (*Bb*) queens into test colonies that had been dequeened 48 h previously. Survival of the three introduced queens was recorded after 24 h. At this time, any surviving queens were removed and, after a further 6 h in queenless condition, the original adoptive queen was returned to the experimental colonies. The assay for the MQPW treatment was modified from that for the PQMW treatment because preliminary tests showed that queenright colonies of the former type almost always reject any introduced queens. As a check for possible unanticipated effects of the modified assay procedure, four of the PQMW colonies were assayed using this procedure as well as the original procedure at four time points; results were identical between the two procedures for each of these colonies at every time point (data not shown), indicating that variation in the assay procedures did not hamper our ability to diagnose colony social organization. The introduced queens in all assays were placed in the foraging area of the experimental colonies' rearing trays while avoiding undue physical disturbance.

Assays of the PQMW colonies were conducted four times over the course of the study: immediately before adoption of new queens, and at approximately 40, 69, and 114 days after adoption (see Appendix for specific sampling times for each colony). Assays of the MQPW colonies were conducted three times: just before queen adoption, and at approximately 75 and 129 days after adoption. Colony MQPW6 was assayed a fourth time as well, at 162 days, because it alone among colonies in this treatment had not rejected all introduced queens by the third assay point.

The proportions of PQMW and MQPW colonies accepting introduced supernumerary *Bb* queens were summarized for each discrete sample interval, using the mean period since adoption of new queens for each colony at each assay point. The 95% confidence intervals around the proportions were estimated by an exact method (Johnson and Kotz 1969) when the proportions were 0 or 1, or by jackknifing over colonies in the other cases.

Worker genotype composition in assayed colonies

At the completion of each assay, the nests were disturbed by lifting their tops and several hundred workers were collected haphazardly among those scattered throughout the foraging area of the rearing tray. The *Gp-9* genotypes of 41–74 (mean=45.6) of these workers were determined, and the 95% confidence intervals around the estimated genotype proportions were obtained using a jackknife procedure as described above. The few workers found to possess the homozygous *bb* genotype (<3% of all workers bearing the *b* allele) were pooled with heterozygous workers for the frequency estimates. For colonies in which only *BB* homozygotes were found in the sample examined, the jackknife 95% confidence intervals were computed by assuming that the next individual to be genotyped would have been a heterozygote.

Results

Results of all assays for colony social organization were unambiguous. For the assays involving queenless colonies (MQPW treatment), multiple introduced *Bb* queens were not all accepted in every case in which any such queens were accepted – that is, there were no instances of only one of the three introduced queens being accepted. For the assays involving queenright colonies (PQMW treatment), the resident queen was invariably retained whenever the single introduced *Bb* queen was accepted. Therefore, the results of the assays unequivo-

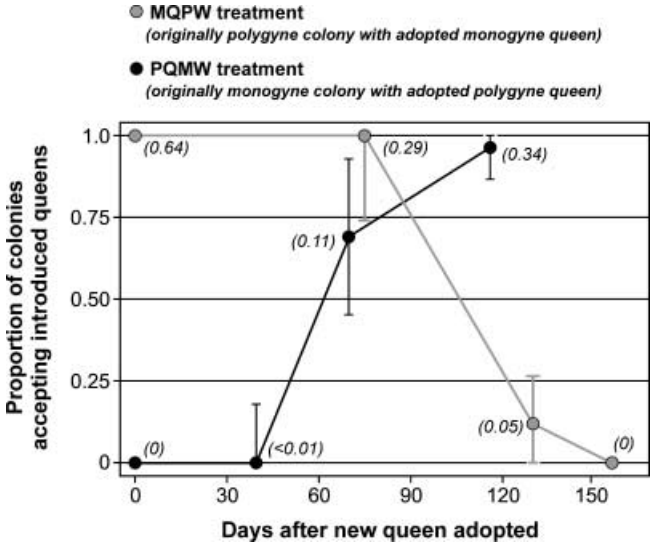

Fig. 1 Mean proportions of MQPW (*n*=11) and PQMW (*n*=15) colonies accepting introduced supernumerary *Gp-9*^{Bb} queens (i.e., displaying polygyne social organization) at varying times following adoption of new queens. *Bars* represent 95% confidence intervals around these mean proportions. The time plotted for each assay point is the mean time since queen adoption for all colonies assayed in each discrete sample interval (see Appendix). Values in *parentheses* indicate the mean proportions of workers with the *Bb* genotype in each class of colonies for each assay point. Colonies were invariant in their acceptance or rejection of supernumerary *Bb* queens immediately before queens were adopted (as is characteristic of unmanipulated colonies of the alternate forms), so no confidence intervals are presented for the day 0 points. Only one MQPW colony was assayed at day 162. The raw data summarized here are presented in the Appendix

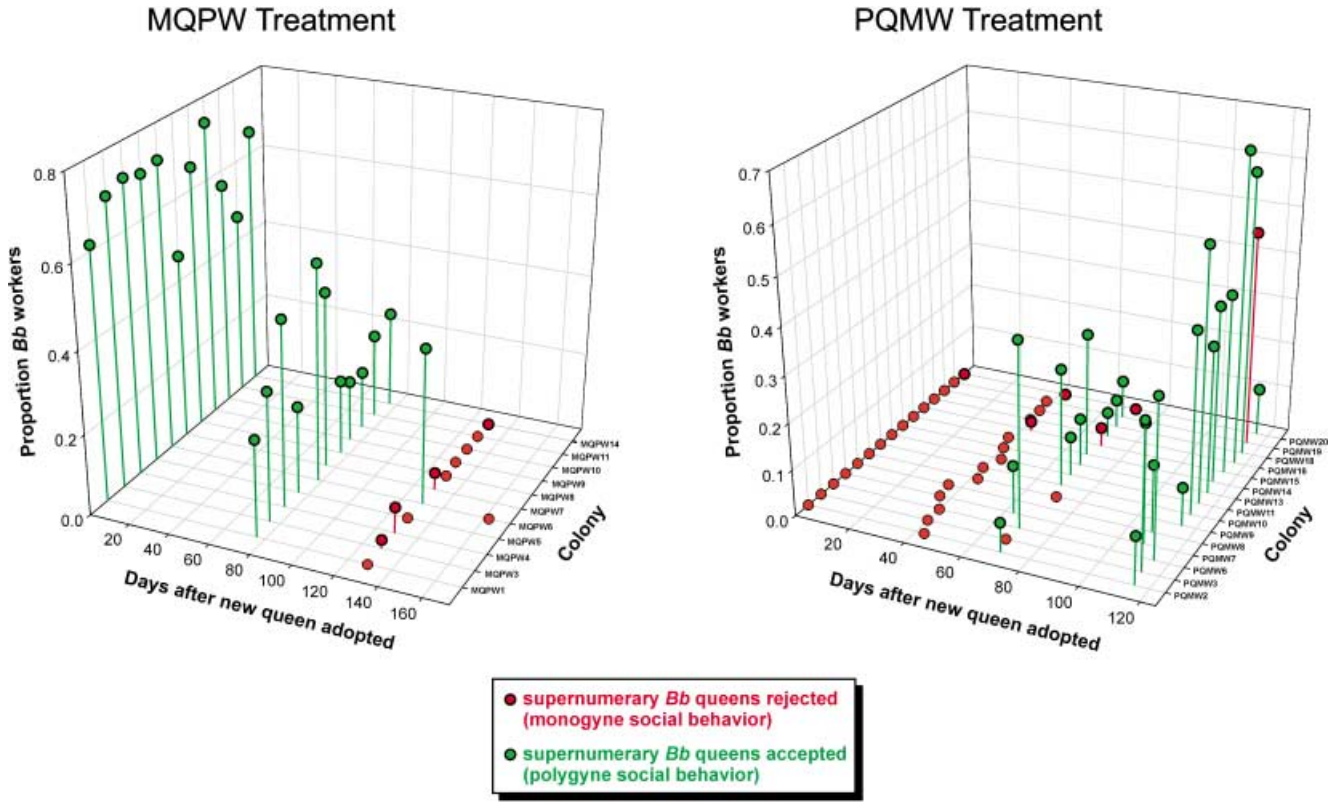

Fig. 2 Estimated *Gp-9* genotype compositions for adult workers and results of assays for colony social organization for individual experimental colonies at varying times following adoption of new queens

cally reveal whether or not a colony was willing to tolerate multiple heterozygous queens at a given point, and thus, whether the colony exhibited the polygyne or monogyne behavioral phenotype.

Assay results are summarized in Fig. 1. All MQPW colonies acted like the typical polygyne colonies from which they were derived for at least 74 days after having received substitute monogyne queens, invariably accepting multiple introduced heterozygous queens through the second assay point. However, by the third assay point at around 129 days into the experiment, 10 of the 11 MQPW colonies (91%) had converted to monogyne social behavior, as shown by their rejection of introduced queens. After another 32 days, the final colony in this treatment also converted to monogyny. The cumulative conversion of the MQPW test colonies from polygyny to monogyny paralleled the decrease through time in the estimated average frequency of adult workers with genotype $Gp\text{-}9^{Bb}$ (Fig. 1). This pattern is expected under the simple null hypothesis that worker *Gp-9* genotype composition in a colony determines social organization and, more specifically, that some minimal frequency of workers bearing the *b* allele must be present for the polygyne social phenotype to be expressed.

All PQMW colonies acted like the typical monogyne colonies from which they originated for at least 35 days after receiving their replacement polygyne queens; they invariably destroyed introduced heterozygous queens through the second assay point (Fig. 1). However, by the third assay point at around 69 days, 10 of the 14 assayed colonies (71%) exhibited a shift in social behavior from monogyny to polygyny, as indicated by their tolerance of multiple heterozygous queens. Effectively all of the PQMW colonies had converted by the final assay point at around 114 days; the only colony that did not accept multiple introduced queens at that point (PQMW19) had accepted them at the previous assay point and so is assumed to represent an example of the rare occurrence of false negatives expected with this treatment/assay (see Discussion). Two patterns in these data are again consistent with the null hypothesis that some minimal frequency of workers bearing the *b* allele must be present for polygyny to be expressed. First, the cumulative conversion of the PQMW test colonies from monogyny to polygyny corresponded with the appearance and increased average frequency of colony workers with the *Bb* genotype through time (Fig. 1). Second, colonies that had already converted by the third assay point contained significantly greater proportions of *Bb* workers than did colonies that had not yet converted at this time (one-tailed Mann-Whitney test, $P<0.003$), with colonies of the latter type containing on average only 1% heterozygous workers (see Appendix).

Even stronger support for the null hypothesis comes from examination of the proportions of heterozygous adult workers in individual colonies in conjunction with the outcome of each assay (Fig. 2; see also Appendix). Every colony in the MQPW treatment showed a monotonic decrease in the proportion of *Bb* workers and a concomitant switch from polygyny to monogyny once

such workers occurred at a frequency of less than 10%. Importantly, the sole MQPW colony that failed to convert to monogyny by the third assay point (MQPW6) had a far higher proportion (38%) of heterozygous workers than this, presumably because of relatively low fecundity of the adoptive queen and a more gradual replacement of the original worker force in this colony.

An inverse, complementary pattern was found for colonies in the PQMW treatment. Every colony displayed a steady increase in the proportion of *Bb* workers after the second assay point, and those that exceeded 5% such workers at the third assay point consistently switched from monogyny to polygyny. The remainder switched to polygyny by the fourth assay point, at which time all contained a worker force with greater than 8% heterozygotes. (The exceptional colony PQMW19, which reverted back to monogyny at the fourth assay point, is the presumed false negative discussed below.) The results from both treatments thus suggest that only a low proportion of *Bb* worker adults need be present in a colony to confer on it the polygyne social organization. That is, there appears to be a "social transition threshold" of no more than 10% heterozygous workers, which represents the minimum frequency of such workers required for the expression of the polygyne social organization in this ant.

Discussion

The results of this study clearly support the hypothesis that colony social organization in *S. invicta* is under strong genetic control, with the presence or absence of workers bearing the *Bb* genotype at the *Gp-9* locus determining whether a colony behaves as a polygyne colony (with multiple reproductive queens) or a monogyne colony (with a single such queen), respectively. Social organization of the experimental colonies studied here changed in a predictable manner after these colonies adopted a single queen of the alternate form. The adopted queens bore *Gp-9* genotypes different from those of the original queens, and thus they produced worker offspring that also differed in *Gp-9* genotype from the original colony members. Through time, experimental colonies that initially contained no *Bb* workers acquired them at increasing frequencies, whereas colonies that initially contained many such workers gradually lost them. As worker *Gp-9* genotype compositions changed, colonies converted to the alternate social organization. Remarkably, these switches consistently occurred when frequencies of *Bb* workers passed an identifiable threshold, such that colonies with fewer than 5% such workers behaved like monogyne colonies and colonies with more than 10% behaved like polygyne colonies. Our data thus confirm the prediction that colony social organization in this ant can be altered by manipulating the frequencies of adult *Bb* workers, and in so doing they validate the specific null hypothesis that the expression of polygyny requires the presence in a colony, at some nominal frequency, of adult workers bearing the *b* allele.

A possible, though unlikely, alternative explanation for our results is that the changeovers in colony social organization occurred because workers became increasingly habituated to the chemical signature of the queen that they adopted. As a result, they may have become increasingly tolerant of queens of the same *Gp-9* genotype and intolerant of queens of the alternate genotype that were introduced [see Keller and Ross (1998) for evidence that workers use cuticular chemical cues to distinguish queens of different *Gp-9* genotypes]. This explanation ignores the fact that no experimental colonies had switched social organization by the second assay point despite having experienced their adoptive queens for at least 35 days (PQMW colonies) or 74 days (MQPW colonies), periods of time known to be more than ample for such habituation to have occurred (L. Keller and K. Ross, unpublished data). Moreover, five colonies still had not changed social organization by the third sample point [these colonies, however, had the highest (MQPW treatment) and lowest (PQMW treatment) frequencies of heterozygous workers at that point, as expected under the null hypothesis]. Finally, the experiments of Ross and Keller (1998) showed that colonies founded by single *BB* queens mated to *b* males, and so containing only heterozygous workers, readily accepted multiple heterozygous queens introduced 6 months after colony founding, again suggesting that habituation to the mother queen's chemical profile cannot explain subsequent patterns of worker tolerance toward queens.

The results of this study are of special interest because they provide an estimate of the minimum proportion of a colony's worker force that must be heterozygous at *Gp-9* in order that the colony display polygyne social behavior (the genotype frequency threshold for transition in social organization). The null hypothesis makes no predictions in this regard because polygyne colonies in the wild and those used in previous laboratory tests of the hypothesis invariably contained high frequencies of heterozygous workers (see Keller and Ross 1998; Ross and Keller 1998; also Appendix). The surprisingly low value of no more than 10% that we estimate for this threshold suggests that only relatively few heterozygotes need be present in a colony to confer the polygyne social phenotype to the entire group. This is an example of a dominant behavioral effect at the level of the colony, whereby only a fraction of workers need express a particular behavior in order that the consequences be manifested at the group level (e.g., Trump et al. 1967; Craig 1980). It seems straightforward to explain the proximate basis of such behavioral dominance when destruction of individuals is involved, as in the rejection of *BB* queens by polygyne colonies (Keller and Ross 1998) or in the destruction of sexual brood to manipulate sex ratios (Aron et al. 1995), because very few workers are sufficient to mortally wound a sexual. However, the proximate mechanism by which infrequently occurring *Bb* workers can *prevent* the execution of introduced *Bb* queens is not clear. These workers are unlikely to physically surround and protect the queens from attack, as such interference among workers has not been ob-

served. Rather, heterozygous workers may influence the behavior of other colony workers toward introduced queens, for example, by recognizing the queens and releasing an anti-aggression pheromone in response. Alternatively, heterozygous workers may carry the same chemical cues that distinguish *Bb* from *BB* queens, so that the presence of even a low frequency of these workers habituates the entire worker force to introduced *Bb* queens. Further behavioral and pheromonal studies are required to clarify the specific mechanisms involved in worker acceptance of potential reproductive queens (e.g., Seeley 1979; Sorensen et al. 1985; Chen and Vinson 1999; Vargo and Hulsey 2000), with the goal of understanding how the summed actions of relatively few individual workers can cause mass (colony-level) effects with respect to regulation of queen number and social organization.

The phenomenon of dominant behavioral effects at the group level represented by tolerance of multiple *Bb* queens in polygyne colonies can be viewed also in the somewhat different context of indirect genetic effects – that is, the influence of the social environment on the behavior of colony members when this social environment is itself altered by the expression of specific genes in some other colony members (Wolf et al. 1998). Workers with the heterozygous genotype at *Gp-9* presumably alter the colony social environment in a manner causing homozygous *BB* workers to become tolerant of multiple heterozygous queens, as discussed above. The evolution of *Gp-9* as a genetic element regulating fundamental social attributes can potentially be profitably modeled within the theoretical framework of indirect genetic effects, although better understanding of the mechanistic, behavioral bases of worker regulation of queen number would presumably help this effort.

Our estimate of the proportion of heterozygous workers that represents the social transition threshold may be subject to several potential sources of error. First, because of the moderate sample sizes used to estimate worker genotype frequencies at each assay point, each estimate has substantial 95% confidence intervals associated with it and so is relatively imprecise (Appendix). Nonetheless, these individual estimates, when taken across colonies and sample points, reveal consistent switches in social organization when colony heterozygote frequencies cross a threshold between 5–10%. Second, the individual colony estimates must be biased in many cases because of the course temporal resolution of the assays. That is, some PQMW colonies may have switched to polygyny up to several weeks earlier than was recorded (and at correspondingly lower heterozygote frequencies), and some MQPW colonies would have been observed to switched to monogyny at higher heterozygote frequencies had they been assayed and sampled more frequently. However, the large number of replicate colonies makes it likely that some in each treatment were assayed at a time when they were near the transition threshold. Third, the fact that *Gp-9* genotype is weakly but significantly associated with worker size (Goodisman et al. 1999), combined with the fact that the very smallest workers cannot be genotyped with the protein-based procedure we employed here, may have contributed an upward bias to our estimates for both treatments. Finally, the methods we used to assay the experimental colonies and obtain worker samples may have led to some bias in the estimates of the transition threshold. In *S. invicta*, worker age is associated with task preference and location with respect to the brood with younger workers more likely to be involved in tending brood within the nest, and older workers more likely to be foraging away from the nest (Mirenda and Vinson 1981). We assayed colonies by introducing queens into the foraging areas, where they would be encountered initially by foragers, but we sampled workers from the entire colony to obtain a general estimate of worker genotype compositions. Behavioral observations suggest that aggression toward queens that culminates in execution takes place in the foraging arena if the queens are introduced there (e.g., Keller and Ross 1993, 1998), but other behavioral components in the potentially complex sequences leading to eventual acceptance or rejection likely take place within the brood chambers, where reproductive queens ultimately take up residence. Without additional detailed information on the processes of queen rejection and acceptance, it remains to be seen whether our samples used to estimate proportions of *Bb* workers were obtained in the most relevant way for defining the social transition threshold.

One exceptional data point in this study is represented by the reversion of colony PQMW19 from polygyne back to monogyne behavior at the final assay point (see Fig. 2). While this reversion may be attributed to the inevitable noise expected in simple assays of complex biological systems, it more likely stems from some level of imprinting by workers on their queen whenever only a single queen is present in the colony (an abnormal circumstance for colonies with *Bb* workers), which can lead to the rejection of any supernumerary queens that are introduced. This effect was detected in an earlier study (unpublished data from Ross and Keller 1998), in which there was a low level of rejection of supernumerary *Bb* queens by colonies with *Bb* workers when a single reproductive queen was present (false negatives), compared to the complete acceptance observed when such test colonies were held queenless for 3 days (which presumably abolished the imprinting recognition template required for subsequent discrimination). This effect seems especially strong when the single resident queen has the *BB* genotype (L. Keller and K.G. Ross, unpublished data), obliging us in the present study to use a modified assay in which resident queens were removed prior to queen introductions into MQPW colonies.

In conclusion, this study confirms a strong genetic component to social organization in *S. invicta*, whereby the presence of workers that are heterozygous at *Gp-9* confers the polygyne social organization upon a colony while the absence of such workers leads to the expression of the monogyne social system. Moreover, our results point to a threshold frequency of around 5–10% heterozygous workers, which when crossed leads to a transition in colony social organization. Detailed investigations of the sequence of events involved in worker acceptance or rejection of potential reproductive queens in the colony, which constitutes

the proximate mechanism of regulation of social organization, are necessary to shed light on the means by which relatively few workers can prescribe the emergent social system of a large and complex society. Knowledge of the gene product of *Gp-9* and the pathways in which it functions should complement information on the behaviors and pheromones involved in worker regulation of queen number to yield a more comprehensive account of the evolution of colony social organization in fire ants.

Acknowledgements We thank Erin Cox for technical assistance, Mark Mescher for input during the study, and Michael Krieger for comments on an earlier draft of the manuscript. This study was supported in part by funds from the National Geographic Society, the Georgia Agricultural Experiment Stations (University of Georgia), the U.S. National Science Foundation, and the Swiss National Science Foundation.

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Appendix

Worker *Gp-9* genotype compositions in experimental colonies (with jackknife 95% confidence intervals) and results of assays for colony social organization. Italics indicate assay points at which colonies rejected introduced supernumerary *Bb* queens (behaved as monogyne colonies), whereas the absence of italics indicates assay points at which colonies accepted such queens (behaved as polygyne colonies). Sample size is the number of workers genotyped at *Gp-9*. The few workers with the *bb* genotype found in this study are pooled with heterozygotes. Source colonies for the PQMW experimental colonies were monogyne and thus would not be expected to contain *Bb* workers, as supported also by the absence of such workers among the 15 genotyped from each colony at the onset of the experiment

Treatment	Colony	Period since adoption of queen (days)	Sample size	Proportion of workers with genotype *Bb*	95% confidence interval		Period since adoption of queen (days)	Sample size	Proportion of workers with genotype *Bb*	95% confidence interval	
					Lower	Upper				Lower	Upper
MQPW	1	0	26	0.615	0.425	0.806	76	42	0.238	0.108	0.369
	3	0	40	0.700	0.556	0.844	75	47	0.319	0.184	0.454
	4	0	39	0.718	0.575	0.861	75	48	0.458	0.316	0.601
	5	0	44	0.705	0.568	0.841	75	46	0.217	0.097	0.338
	6	0	28	0.714	0.544	0.885	78	41	0.537	0.382	0.691
	7	0	26	0.462	0.266	0.657	75	48	0.438	0.296	0.579
	8	0	26	0.654	0.467	0.840	76	48	0.188	0.076	0.299
	9	0	42	0.738	0.604	0.873	74	46	0.152	0.047	0.257
	10	0	48	0.563	0.421	0.704	74	48	0.146	0.045	0.247
	11	0	35	0.459	0.297	0.622	74	47	0.213	0.095	0.331
	14	0	49	0.653	0.518	0.788	76	41	0.244	0.111	0.377
Mean prop. *Bb* in colonies accepting supernum. queens:				0.635					0.286		
Mean prop. *Bb* in colonies rejecting supernum. queens:				–					0.286		
PQMW	2	*0*	*15*	*0*			*43*	*48*	*0*	*0.000*	*0.060*
	3	*0*	*15*	*0*			*40*	*48*	*0*	*0.000*	*0.060*
		0	*15*	*0*			*40*	*48*	*0*	*0.000*	*0.060*
	7	*0*	*15*	*0*			*36*	*48*	*0*	*0.000*	*0.060*
	8	*0*	*15*	*0*			*35*	*48*	*0*	*0.000*	*0.060*
	9	*0*	*15*	*0*			*42*	*48*	*0*	*0.000*	*0.060*
	10	*0*	*15*	*0*			*40*	*48*	*0*	*0.000*	*0.060*
	11	*0*	*15*	*0*			*43*	*48*	*0*	*0.000*	*0.060*
	13	*0*	*15*	*0*			*40*	*47*	*0*	*0.000*	*0.062*
	14	*0*	*15*	*0*			*38*	*48*	*0*	*0.000*	*0.060*
	15	*0*	*15*	*0*			*43*	*47*	*0.021*	*0.001*	*0.063*
	16	*0*	*15*	*0*			*39*	*48*	*0*	*0.000*	*0.060*
	18	*0*	*15*	*0*			*39*	*48*	*0*	*0.000*	*0.060*
	19	*0*	*15*	*0*			*38*	*48*	*0*	*0.000*	*0.060*
	20	*0*	*15*	*0*			*42*	*47*	*0*	*0.000*	*0.062*
Mean prop. *Bb* in colonies accepting supernum. queens:				0.000					0.001		
Mean prop. *Bb* in colonies rejecting supernum. queens:				0.000					0.001		

[a] Colony MQPW6 was assayed at one time point beyond the other colonies in this treatment in order to track the predicted switch in social organization
[b] Experimenial colony PQMW8 was not assayed at this time point

Period since adoption of queen (days)	Sample size	Proportion of workers with genotype *Bb*		95% confidence interval		Period since adoption of queen (days)	Sample size	Proportion of workers with genotype *Bb*	95% confidence interval	
				Lower	Upper				Lower	Upper
129	*48*	*0*	*0.000*	*0.060*						
129	*46*	*0.022*	*0.001*	*0.064*						
129	*62*	*0.065*	*0.003*	*0.126*						
129	*45*	*0*	*0.000*	*0.064*						
130	47	0.383	0.243	0.523	*162*[a]	*46*	*0*	*0.000*	*0.063*	
130	*46*	*0.043*	*0.001*	*0.103*						
130	48	*0*	*0.000*	*0.060*						
129	*48*	*0*	*0.000*	*0.060*						
129	*48*	*0*	*0.000*	*0.060*						
129	*46*	*0*	*0.000*	*0.063*						
129	*48*	*0*	*0.000*	*0.060*						
		0.383					–			
		0.013					0			
70	47	0.064	0.001	0.135	115	48	0.104	0.017	0.192	
68	*48*	*0*	*0.000*	*0.060*	114	48	0.313	0.180	0.445	
69	43	0.395	0.248	0.543	115	47	0.340	0.204	0.477	
63	48	0.104	0.017	0.192	108	47	0.255	0.129	0.381	
–[b]	–	–	–	–	108	48	0.146	0.045	0.247	
71	*48*	*0*	*0.000*	*0.060*	115	48	0.083	0.004	0.162	
69	47	0.255	0.129	0.381	115	46	0.391	0.249	0.534	
69	47	0.085	0.005	0.166	115	46	0.543	0.398	0.689	
69	48	0.104	0.017	0.192	115	47	0.319	0.184	0.454	
68	48	0.271	0.144	0.398	114	47	0.383	0.243	0.524	
70	*48*	*0.042*	*0.001*	*0.099*	115	54	0.389	0.258	0.520	
69	74	0.054	0.002	0.106	116	45	0.667	0.527	0.806	
69	48	0.063	0.001	0.132	116	46	0.609	0.466	0.751	
68	47	0.085	0.005	0.166	*115*	*47*	*0.468*	*0.324*	*0.612*	
70	*66*	*0*	*0.000*	*0.044*	116	48	0.104	0.017	0.192	
		0.148					0.332			
		0.010					–[c]			

[c] Colony PQMW19 is excluded from consideration here because it reverted from accepting a supernumerary queen in the previous assay point to rejecting one at this assay point (see text)