Behavioral Discrimination Between Monogyne and Polygyne Red Fire Ants (Hymenoptera: Formicidae) in Their Native Range

MÓNICA G. CHIRINO,[1,2] LAWRENCE E. GILBERT,[3] AND PATRICIA J. FOLGARAIT[1]

Ann. Entomol. Soc. Am. 105(5): 740–745 (2012); DOI: http://dx.doi.org/10.1603/AN11073

ABSTRACT Nestmate recognition among social insects is presumed to restrict non-nestmates from exploiting nest resources. Here, we developed aggression bioassays to assess the discrimination behaviors of both polygynous and monogynous forms of the red fire ant, *Solenopsis invicta* Buren, during symmetrical interactions in neutral arenas. Workers from polygyne colonies exhibited risk avoidance behaviors; that is, defensive postures or the avoidance of direct contact during interactions. Workers from monogyne colonies always exhibited aggressive behaviors in the form of physical or chemical attacks. In interactions between both, monogyne workers usually started the aggression by surrounding and biting the polygyne ants. Polygyne *S. invicta* workers also distinguished nestmates from foreigners, but their response was not as aggressive as that of monogynes. The proposed ethogram that we constructed identified monogyne and polygyne forms of *S. invicta* colonies in concordance with current measures, including number of queens, and expression of the *Gp-9* gene.

KEY WORDS recognition, social form, *Solenopsis invicta*, Argentina

Recognition between conspecifics is an essential attribute of ant social behavior for repelling non-nestmates and protecting food resources (Hölldobler and Wilson 1990). Red fire ants, *Solenopsis invicta* Buren, use olfactory cues produced by queens to discriminate between colony members and conspecific intruders (Vander Meer and Alonso 2002, Vander Meer et al. 2008). *Solenopsis invicta* also uses environmentally derived cues to discriminate between colony members and nonmembers (Obin et al. 1993). Red fire ants have two distinct forms of colony organization: monogyny and polygyny, distinguishable by the number of reproductive queens (Ross and Keller 1995), how reproduction is divided among members of the colony, the number of individuals produced, the degree of genetic relatedness, and queens' and workers' behaviors (Goodisman and Ross 1997, Keller and Ross 1998, Adams and Balas 1999, Gotzek and Ross 2007). Different behaviors are correlated with allelic differences at the nuclear gene *General Protein-9* (*Gp-9*) that codes for two groups of odor binding proteins (Krieger and Ross 2002). Queens of monogyne colonies possess *B*-like alleles (with *BB* genotype) and are more prolific, heavier, and longer-lived than queens of polygyne colonies (Keller and Ross 1999, Gotzek and Ross 2007). In Argentina, polygyne colonies can be heterozygous (*Bb*) or homozygous (*BB*), thus some polygyne workers present *b*-like alleles (Ross et al. 1996, Ross 1997, Krieger and Ross 2002). Monogyne workers kill foreign queens and aggressively defend their territory (Keller and Ross 1998). However, not all behaviors are universal, primarily because worker behaviors depend on the ecological context in which they develop (Keller and Ross 1993, Obin et al. 1993, Vander Meer and Alonso 2002, Goodisman et al. 2007), and the manipulation of worker genotypes can elicit change in behaviors (Ross and Keller 2002, Gotzek and Ross 2008). Therefore, behaviors of native populations can differ from those of introduced populations.

Nestmate recognition in *S. invicta* has been studied extensively in the past. However, previous bioassays mainly consisted of introducing alien workers within several colonies (Obin and Vander Meer 1988, Morel et al. 1990, Obin et al. 1993, Vander Meer and Alonso 2002, Vander Meer et al. 2008) without considering that each colony possesses its own exclusive foraging territory (Showler et al. 1990, Adams and Tschinkel 2001). Objectives of this study were to assess the aggressive behavior of monogyne and polygyne red fire ant workers by studying interaction in neutral arenas, and to develop a reliable ethogram for readily distinguishing between monogyne and polygyne colonies of *S. invicta* in the field.

Materials and Methods

Collection and Maintenance of Ants. A red fire ant mound was considered a "colony" and all mounds studied were separated from others by >2 m. Ants from monogyne and polygyne populations were collected at two field sites in Argentina: Parque Nacional

---

[1] Centro de Estudios e Investigaciones, Universidad Nacional de Quilmes, Roque Saenz Peña 352, Bernal (B1876BXD), Buenos Aires, Argentina.

[2] Corresponding author, e-mail: mchirino@unq.edu.ar.

[3] Section of Integrative Biology and Brackenridge Field Laboratory, University of Texas, Austin, TX 78712.

0013-8746/12/0740–0745$04.00/0 © 2012 Entomological Society of America

Table 1. Ethogram for assessing levels of aggression within and between monogyne and polygyne *S. invicta* workers by behavioral responses (modified from Obin and Vander Meer 1988)

Category	Level	Behavioral observations
Tolerance	1[a]	Rest: ants alone or in groups remain motionless or clean their antennae.
Tolerance	2	Recognition: ants walk or exhibit rapid antennation towards other ants for >5 s.
Risk avoidance	3[b]	Stereotypical postures: ants with mandibles held open and exhibiting "C" posture (gaster under body), or curled body.
Risk avoidance	4	Avoid contact: ants with open mandibles evade or sidle away from other ants; sometimes short pursuits and antennations for <5 s.
Aggression	5	Physical or chemical threat: ants attempt to bite, or gasters elevated, vibrating and emitting venom[c].
Aggression	6	Attack: 1 or 2 ants bite, sting, or dismember antennae or legs of opponent.

[a] Postures from Cassill et al. (2009).

[b] Postures from Wuellner et al. (2002).

[c] "Gaster flagging" behavior (Obin and Vander Meer 1985).

Chaco (26° 48′ S and 59° 34′ W), Chaco Province, and near San Javier city (30° 35′ S and 59° 55′ W), Santa Fe Province. *S. invicta* populations occur in pastures (grassy patches) and along natural trails throughout forests, both in subtropical forests in the Chaqueña Phytogeographic Province and the Espinal Phytogeographic Province, respectively (Cabrera and Willink 1980). Field observations were conducted between February and October 2005 and between November 2006 and October 2007. Ants were identified according to the key provided by Pitts et al. (2005).

Three types of measurements were taken in the field as well as in the laboratory from each of six monogynous and six polygynous mounds at both sites. Colonies were excavated and separated from the soil by means of a dripping system (Banks et al. 1981). Clumped mounds separated by <1 m were considered as being the same polydomic nest; only the largest mound of each was excavated. In the laboratory, the colonies were placed in 30 × 21 × 7.5 cm plastic boxes coated with Fluon (polytetrafluoroethylene, Asahi Glass Flouropolymers, Exton, PA) to prevent escape. Colonies were placed in a rearing room at 28 ± 1°C, a photoperiod of 12:12 (L:D) h and 80 ± 10% RH. Ants were fed 50:50 sugar:water solution, pure water, and three fresh crickets, *Acheta domesticus* L., three times each week. Monogynous and polygynous forms were determined by counting the numbers of wingless queens found in excavated mounds. Queens from the collected nests were confirmed to be inseminated when they produced workers in the laboratory. Mounds with two or more functional queens were considered polygynous. The social form of mounds with one queen or no queens was determined by the method of Valles and Porter (2003). Protein extracts pooled from 15 to 20 workers per colony were assayed for the presence of the *B* allelic protein, and colonies exhibiting only the amplification of the *Gp-9B* allele were considered to be monogynous.

Qualitative Behavior Field Assays. To assess monogyne and polygyne forms of *S. invicta,* behavioral interactions of workers belonging to the same nest (control) were compared with interactions with nonnestmates from neighboring colonies in the field. Ants were collected with soft forceps and put in arenas (plastic boxes coated with Fluon, 16 × 7.5 × 5 cm). Ants from each colony remained in a separate arena for 30 min, to allow ants to become accustomed to the container (new environment). Afterwards, pairs chosen for interactions were placed simultaneously into a new arena. Interactions were measured among eight workers (four per nest), and levels of aggression were determined by counting the numbers of tolerant and aggressive interactions (Table 1) during 10 min. The social organization of each colony was assessed on the basis of nest dominance per grassy patch, expressed as the percentage of monogynous and polygynous colonies. Additionally, the distance from each mound to the nearest mound was recorded. The colonies were then excavated and taken to the laboratory.

Quantitative Behavior Laboratory Assays. To assess both monogyne and polygyne forms of *S. invicta* colonies, behavioral comparisons were made between forager (head width: 0.95 ± 0.04 mm) nestmate ants (control) and between workers from different colonies in neutral arenas. Arenas were either used one time or reused after cleaning with a 10% hypochlorite solution for 24 h. Ants were kept in separate containers for 30 min before release into the arena to test monogyne-monogyne, polygyne-polygyne, and monogyne-polygyne combinations using four per colony. Gasters were painted to differentiate social organizations as they were being observed (Markal, LA-CO Industries Inc., Elk Grove, IL). All ants were used once to avoid behaviors related to familiarity. Interactions were recorded during the first 60 s, and then every 30 s for the following 9 min. Averaged index scores of ant behaviors were used to rank the assay combinations into six ranks of aggression that included three behavioral categories: tolerance, risk avoidance, and aggression (Table 1). During interactions between monogyne versus polygyne ants the social organization type to attack first and the reaction of the attacked ants were recorded.

Statistical Analyses. Minimum mound distances between colonies and densities were analyzed using two-tailed analysis of variance (ANOVA) (ANOVAs, site, and social organization). For adjusting significance levels to control type I error ($\alpha = 0.05$) in multiple comparisons made for workers' behaviors, a posteriori contrasts were performed using the Fisher procedure ($P < 0.017$; Sokal and Rolhf 1969, SAS Institute 1998).

Table 2. Characteristics recorded among red fire ant mounds under field conditions: min. distance (m), and percent density (%) of monogyne mounds, and predominating ant behavior observed ($N = 3$) during interactions

Site	Mound	Minimum distance (m)	% monogyne per patch	Level index, % occurrence[a]	Category	Predicted social organization
Chaco	1	0.83	72.7	Walk, 86.7	Tolerance	Polygyny
	2	5.31	72.7	Bite-sting, 66.7	Aggression	Monogyny
	3	5.31	72.7	Bite-sting, 100	Aggression	Monogyny
	4	1.26	72.7	Antennation, 93.3	Tolerance	Polygyny
	5	0.92	80.0	Walk, 100	Tolerance	Polygyny
	6	1.16	80.0	Chemical threat, 46.7	Aggression	Monogyny
	7	2.65	84.6	Pull members, 46.7	Aggression	Monogyny
	8	1.12	84.6	Rest in group, 76.7	Tolerance	Polygyny
	9	1.61	84.6	Rest, 50.0	Tolerance	Polygyny
	10	2.13	84.6	Walk, 70.0	Tolerance	Polygyny
	11	4.31	84.6	Pull members, 40.0	Aggression	Monogyny
	12	5.36	84.6	Dismember-dead ants, 60.0	Aggression	Monogyny
San Javier	1	3.83	70.0	Bite-sting, 76.7	Aggression	Monogyny
	2	0.66	70.0	Walk, 66.7	Tolerance	Polygyny
	3	1.26	70.0	Dead ants, 46.7	Aggression	Monogyny
	4	1.00	70.0	Rest, 66.7	Tolerance	Polygyny
	5	0.32	59.3	Walk, 83.3	Tolerance	Polygyny
	6	1.26	59.3	Antennation, 50.0	Tolerance	Polygyny
	7	1.60	59.3	Bite, 33.3	Aggression	Monogyny
	8	3.47	59.3	Pull members, 70.0	Aggression	Monogyny
	9	0.51	79.0	Rest, 63.3	Tolerance	Polygyny
	10	0.64	79.0	Walk, 73.3	Tolerance	Polygyny
	11	3.26	79.0	Chemical threat, 53.3	Aggression	Monogyny
	12	5.11	79.0	Bite, 60.0	Aggression	Monogyny

A patch is a grass-dominated area where red fire ant colonies tend to be clustered.

[a] See Table 1 for level descriptions.

Data of behavioral categories obtained from the ethogram and the specific interactions between monogyne and polygyne colonies were analyzed using the Kruskall–Wallis ANOVAs on ranks for global comparisons ($P = 0.025$) and by followed by Mann–Whitney U tests for contrasts between treatments (Daniel 1990, SAS Institute 1998). Behavioral data discriminated by site were analyzed using the Mann–Whitney U test ($P = 0.05$) (SAS Institute 1998).

Results

Qualitative Behavior Field Assays. The most commonly observed behavior in the control treatment was walking, whereas the other combinations resulted in a wider variety of behaviors. Usually polygyne workers were not aggressive toward alien conspecific ants from neighboring mounds. Monogyne workers bit and stung other ants, and vibrated their elevated gasters (Table 2). Minimum mound distances from other nests were shorter for polygynes (1.31 ± 0.14 m) than monogynes (4.02 ± 0.50 m) ($F = 35.74$; df = 1, 20; $P < 0.001$). Mound densities within sampled patches were higher, 74.3%, for monogynes than for polygynes ($F = 28.12$; df = 1, 20; $P = 0.0019$). Minimum mound distances between nests and densities within sampled patches were associated with the final assignment of the social form to the colonies tested (Table 2).

Quantitative Behavior Laboratory Assays. Organization structure affected ant behaviors ($H = 87.78$; df = 3, 123; $P < 0.0001$; Fig. 1) but a site effect was not detected. Eighty-six percent of monogyne workers were aggressive toward foreign ants of either monogyne or polygyne colonies. Polygyne workers recognized nestmate workers versus alien workers and the 80% of them were not aggressive toward foreign polygyne workers by exhibiting risk avoidance behaviors. In the monogyne versus polygyne interactions, the index scores reflected aggression (Fig. 1). Interactions between monogyne and polygyne ants also showed 42.6% risk avoidance behaviors and 50% aggression (Fig. 1). Of 91.7% assays, classification of each colony's corresponded with the quantitative behavior classification in every instance excluding one where we were not able to amplify either alleles (colony five from San Javier).

Aggressive interactions were typified by recognition antennations from monogyne workers on the head and gaster of polygyne workers before the po-

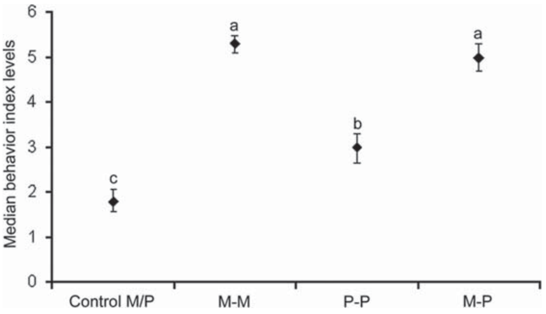

Fig. 1. Median with first and third quartile scores of levels of behavior observed during the interactions within and between monogyne (M) and polygyne (P) *S. invicta* workers. Different letters indicate significant differences ($P < 0.05$, Kruskal–Wallis test); behavioral interactions categories are tolerance, risk avoidance, and aggression.

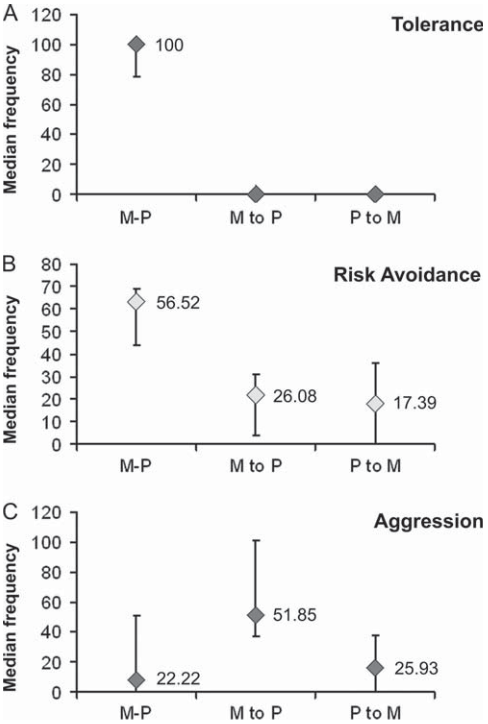

Fig. 2. Median with first and third quartile behavior index levels for interactions between monogyne (M) and polygyne (P) *S. invicta* workers. (A) Tolerance. (B) Risk avoidance. (C) Aggression. *M-P* indicates that both monogyne and polygyne workers showed the same behavior simultaneously. Different letters indicate significant differences ($P < 0.05$). Percentages of interactions by each category of behavior are indicated.

lygyne ants were surrounded and bitten on the postpetiole. Before attacks, the polygyne targeted ants usually displayed no reaction to the monogyne aggressive ants, and attacked polygyne workers did not respond aggressively.

Of all interactions observed between monogyne and polygyne workers, only four exhibited tolerance (Fig. 2a). Monogyne and polygyne workers often avoided the mutual contact through risk avoidance behaviors of which a 26.1% were initiated by a monogyne ant and 17.4% by a polygyne ant (Fig. 2b; $H = 15.43$; df = 2, 20; $P = 0.004$). During risk avoidance, ants displayed open mandibles and curled their bodies. Most, 66.7%, of aggressive behaviors were initiated by monogyne workers, although 33.3% polygyne workers were aggressive (Fig. 2c; $H = 10.57$; df = 2, 20; $P = 0.0051$).

Discussion

The ability of social insects to recognize nestmates from non-nestmates is a fundamental trait for maintaining the integrity of individual colonies (Hölldobler and Wilson 1990). Our study shows that monogyne and polygyne workers of *S. invicta* discriminate between nestmates and foreigners as indicated by different behaviors ranging from tolerance to aggression. Monogyne ants always attacked foreign ants independently if they were from monogyne or polygyne colonies, whereas polygyne ants recognized (but did not attack) foreign polygyne ants, mainly by exhibiting postures similar to behaviors assumed after attacks by *Pseudacteon* phorids (Wuellner et al. 2002). Hostile versus warning behaviors were strongly dependent on the social structure of workers. Therefore, the behavior toward foreign workers was a reliable ethological indicator to characterize monogyne and polygyne colonies of *S. invicta.*

Monogyne *S. invicta* colony territorial area and the mound size are positively correlated (Tschinkel et al. 1995), which, in turn, is regulated by the size (number and biomass of workers) and distance from neighboring colonies (Showler et al. 1990), by their prey density (Showler et al. 1989), and by the colony's collective competitive ability (Adams 2003). In contrast, nestmate discrimination among polygyne colonies is more relaxed (Morel et al. 1990) as workers tolerate conspecific ants alien to the colony, accept other heterozygote queens, and do not aggressively protect their territory from polygyne conspecifics (Keller and Ross 1998). These colonies might increase their reproductive output as a result of having many queens and the possibility of exploiting greater territories by means of cooperative recruitment and interconnected mounds (Bhatkar and Vinson 1987; Morel et al. 1990). Therefore, polygyne workers displayed low aggressive responses toward polygyne non-nestmates because lower aggression results in higher survival. Consequently, the behavior of workers is another reliable factor to characterize both monogyne and polygyne colonies of *S. invicta* (Morel et al. 1990), in addition to considering mean worker sizes, density or distance between mounds, number of queens, or molecular assays (Greenberg et al. 1985; Ross et al. 1997; Porter 1992, 1993; Tschinkel et al. 1995; Goodisman et al. 1999; Adams and Tschinkel 2001; Krieger and Ross 2002).

The ethogram has the advantages of evaluating the behavior of ants using small and inexpensive arenas for isolating behavioral responses associated with nest defense, and tolerance. Polymerase chain reaction analysis corroborated our assessments of whether a colony was monogynous or polygynous. Use of the ethogram permits assessment without disturbing, destroying, or excavating the colonies or performing molecular analyses.

Acknowledgments

We thank R. Plowes for his comments and A. T. Showler for his editorial suggestions that greatly improved this paper. This research was supported by grants from the Programa de Investigación en Interacciones Biológicas from Universidad Nacional de Quilmes, the Lee and Ramona Bass Foundation, and the Robert J. Kleberg and Helen C. Kleberg Foundation. M.G.C. and P.J.F. also thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References Cited

Adams, E. S. 2003. Experimental analysis of territory size in a population of the fire ant *Solenopsis invicta.* Behav. Ecol. 14: 32–37.

Adams, E. S., and M. T. Balas. 1999. Worker discrimination among queens in newly founded colonies of the fire ant *Solenopsis invicta.* Behav. Ecol. Sociobiol. 45: 330–338.

Adams, E. S., and R. Tschinkel. 2001. Mechanisms of population regulation in the fire ant *Solenopsis invicta:* an experimental study. J. Anim. Ecol. 70: 355–369.

Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants. Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, New Orleans, LA.

Bhatkar, A. P., and S. B. Vinson. 1987. Colony limits in *Solenopsis invicta* Buren, pp. 599–600. *In* J. Eder and H. Rembold (eds.), Chemistry and Biology of Social Insects. Paperny, Munich, Germany.

Cabrera, A. L., and A. Willink. 1980. Biogeografía de América Latina, 2nd Ed. Monografía 13, Serie de Biología. Organización de los Estados Americanos, Washington, DC.

Cassill, D. L., S. Brown, D. Swick, and G. Yanev. 2009. Polyphasic/sleep episodes in the fire ant, *Solenopsis invicta.* J. Insect Behav. 22: 313–323.

Daniel, W. W. 1990. Applied nonparametric statistics, 2nd ed. PWS- Kent, Devon, United Kingdom.

Goodisman, M.A.D., and K. G. Ross. 1997. Relationship of queen number and queen relatedness in multiple-queen colonies of the fire ant *Solenopsis invicta.* Ecol. Entomol. 22: 150–157.

Goodisman, M.A.D., P. D. Mack, D. E. Pearse, and K. G. Ross. 1999. Effects of a single gene on worker and male body mass in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 92: 563–570.

Goodisman, M.A.D., K. A. Sankovich, and J. L. Kovacks. 2007. Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta.* Biol. Invasions 9: 571–584.

Gotzek, D., and K. G. Ross. 2007. Genetic regulation of colony social organization in fire ants: an integrative overview. Q. Rev. Biol. 82: 201–226.

Gotzek, D., and K. G. Ross. 2008. Experimental conversion of colony social organization in fire ants (*Solenopsis invicta*): worker genotype manipulation in the absence of queens effects. J. Insect Behav. 21: 337–350.

Greenberg, L., D.J.C. Fletcher, and S. B. Vinson. 1985. Differences in worker size and mound distribution in monogynous and polygynous colonies of the fire ant *Solenopsis invicta* Buren. J. Kans. Entomol. Soc. 58: 9–18.

Hölldobler, B., and O. E. Wilson. 1990. The ants. Belknap Press of Harvard University Press, Cambridge, MA.

Keller, L., and K. G. Ross. 1993. Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta.* Behav. Ecol. Sociobiol. 33: 121–129.

Keller, L., and K. G. Ross. 1998. Selfish genes: a green beard in the red fire ant. Nature 394: 573–575.

Keller, L., and K. G. Ross. 1999. Major gene effects on the phenotype and fitness: the relative roles of *Pgm*-3 and *Gp*-9 in introduced populations of the fire ant *Solenopsis invicta.* J. Evolution. Biol. 21: 672–680.

Krieger, M.J.B., and K. G. Ross. 2002. Identification of a major gene regulating complex social behaviour. Science 295: 328–332.

Morel, L., R. K. Vander Meer, and C. S. Lofgren. 1990. Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 83: 642–647.

Obin, M. S., and R. K. Vander Meer. 1985. Gaster flagging by fire ants (*Solenopsis* spp.): functional significance of venom dispersal behavior. J. Chem. Ecol. 11: 1757–1767.

Obin, M. S., and R. K. Vander Meer. 1988. Sources of nestmate recognition in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Anim. Behav. 36: 1361–1370.

Obin, M. S., L. Morel, and R. K. Vander Meer. 1993. Unexpected, well-developed nestmate recognition in laboratory colonies of polygyne imported fire ants (Hymenoptera: Formicidae). J. Insect Behav. 6: 579–589.

Pitts, J. P., J. V. McHugh, and K. G. Ross. 2005. Cladistic analysis of the fire ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Zool. Scr. 34: 493–505.

Porter, S. D. 1992. Frequency and distribution of polygyne fire ants (Hymenoptera: Formicidae) in Florida. Fla. Entomol. 75: 248–257.

Porter, S. D. 1993. Stability of polygyne and monogyne fire ant populations (Hymenoptera: Formicidae: *Solenopsis invicta*) in the United States. J. Econ. Entomol. 86: 1344–1347.

Ross, K. G. 1997. Multilocus evolution in fire ants: effects of selection, gene flow and recombination. Genetics 145: 961–974.

Ross, K. G., and L. Keller. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. Annu. Rev. Ecol. Evol. S. 26: 631–656.

Ross, K. G., and L. Keller. 2002. Experimental conversion of colony social organization by manipulation of worker genotype composition in fire ants (*Solenopsis invicta*). Behav. Ecol. Sociobiol. 51: 287–295.

Ross, K. G., E. L. Vargo, and L. Keller. 1996. Simple genetic basis for important social traits in the fire ant *Solenopsis invicta.* Evolution 50: 2387–2399.

Ross, K. G., M.J.B. Krieger, D. D. Shoemaker, E. L. Vargo, and L. Keller. 1997. Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. Genetics 147: 643–655.

SAS Institute. 1998. StatView pour Windows, version 5.0. SAS Institute, Cary, NC.

Showler, A. T., R. M. Knaus, and T. E. Reagan. 1989. Foraging territoriality of the imported fire ant, *Solenopsis invicta* Buren, in surgarcane as determined by neutron activation analysis. Insectes Soc. 36: 235–239.

Showler, A. T., R. M. Knaus, and T. E. Reagan. 1990. Studies of the territorial dynamics of the red imported fire ant (Hymenoptera: Formicidae). Agric. Ecosyst. Environ. 30: 97–105.

Sokal, R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco, CA.

Tschinkel, W. R., E. S. Adams, and T. Macom. 1995. Territory area and colony size in the fire ant *Solenopsis invicta.* J. Anim. Ecol. 64: 473–480.

Valles, S. M., and S. D. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of *Gp*-9 alleles. Insectes Soc. 50: 199–200.

Vander Meer, R. K., and L. E. Alonso. 2002. Queen primer pheromone affects conspecific fire ant (*Solenopsis invicta*) aggression. Behav. Ecol. Sociobiol. 51: 122–130.

Vander Meer, R. K., C. A. Preston, and A. Hefetz. 2008. Queen regulates biogenic amine level and nestmate recognition in workers of the fire ant, *Solenopsis invicta.* Naturwissenchaften 95: 1155–1158.

Wuellner, C. T., C. G. Dall'Aglio-Holvorcem, W. W. Benson, and L. E. Gilbert. 2002. Phorid fly (Diptera: Phoridae) oviposition behaviour and fire ant (Hymenoptera: Formicidae) reaction to attack differs according to phorid species. Ann. Entomol. Soc. Am. 95: 257–266.

Received 20 April 2011; accepted 4 June 2012.