

Unexpected, Well-Developed Nestmate Recognition in Laboratory Colonies of Polygyne Imported Fire Ants (Hymenoptera: Formicidae)

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*Aggression bioassays were used to investigate nestmate recognition in polygyne laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren. Unlike workers from polygyne field colonies, laboratory-maintained (> 10 weeks) workers exhibited well-developed nestmate recognition. As in monogyne colonies of this species, both heritable and environmentally acquired (diet) odors provided recognition cues and were roughly additive in their effect. Within diet treatments, polygyne colonies responded in a graded fashion to polygyne conspecifics, monogyne conspecifics, and heterospecifics (*S. richteri* Forel), thus suggesting incipient genetic divergence between the two *S. invicta* social forms. Hypotheses to account for the acute intraspecific discrimination observed in the laboratory are presented. Empirical testing of these hypotheses will illuminate ecological constraints and proximate mechanisms underlying the reduced inter-colony discrimination associated with natural polygyne colonies of this and other ant species.*

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INTRODUCTION

Social insect workers discriminate colony members from nonmembers based on a learned "colony odor" (Wilson, 1971; Hölldobler and Michener, 1980; Vander Meer, 1988). This "nestmate recognition" involves "phenotype matching" (Holmes and Sherman 1982), in which colony members compare the odor labels of encountered individuals with a memory template of familiar colony odors (reviews by Gadagakar, 1985; Breed and Bennett, 1987). Olfactory inputs to worker labels can include heritable "discriminators" transferred from the queen(s) or expressed by workers as well as cues acquired from environmental sources (reviews by Hölldobler and Carlin, 1987; Breed and Bennett, 1987). In the Formicidae, nestmate recognition and social structure are intriguingly correlated. Typically, polygyne colonies that occupy multiple adjacent nests (polydomy) exhibit little or no intercolony discrimination compared to monogyne colonies (Hölldobler and Wilson, 1977; review by Breed and Bennett, 1987; Morel *et al.*, 1990). However, some supercolony- and population-level recognition has been reported (Traniello, 1982; Halliday, 1983; Stuart, 1987; Morel *et al.*, unpublished data). The generally accepted proximate explanation for the association of polygyny with reduced nestmate recognition is the *discriminator variability* hypothesis. This suggests that reduced recognition is a consequence of increased within-colony genetic variation in queen- and worker-derived recognition cues that results from the presence of multiple queens or multiple worker matriline/patriline (Hölldobler and Wilson, 1977; Breed and Bennett, 1987; Stuart, 1987). In this scenario, learned worker templates based on familiar queen or nestmate discriminators become sufficiently "wide" to accommodate the colony odor labels of nonnestmates.

The present study examines nestmate recognition in polygyne laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren. The polygyne form is hypothesized to have arisen *de novo* following the introduction of monogyne founder colonies into North America within the last 50 years (Ross *et al.*, 1987b). In the southeastern United States, monogyne and polygyne *S. invicta* are sympatric and "genetically indistinguishable" by allozyme analysis (Ross *et al.*, 1987b), although Dunton (1991) has demonstrated isozyme differences between the two social forms in Texas populations. Mature polygyne colonies are polydomous, with individual nest mounds containing from several to several hundred functional queens (Glancey *et al.*, 1973; Ross *et al.*, 1987b). Within-nest coefficients of relatedness are not significantly greater than 0 (Fletcher and Ross, 1985). Previous studies have demonstrated little or no intercolony discrimination in polygyne fire ants. Mirenda and Vinson (1982) observed that worker forces from polygyne colonies merged with relatively little aggression. Morel *et al.*, (1990) subsequently demonstrated that, unlike monogyne workers

(Obin, 1986; Obin and Vander Meer, 1988), freshly collected workers from polygyne colonies were not aggressive toward conspecifics of either social form.

Here we report the unexpected finding that workers of polygyne colonies maintained in the laboratory can develop a monogyne-like nestmate recognition response. Using diet as an environmental variable, we also demonstrate the contribution of both heritable discriminators and environmental odors to this response. These data are interpreted with respect to the *discriminator variability* hypothesis (above). In addition, we examine between- vs within-population recognition among polygyne colonies, as well as polygyne response to polygyne *S. invicta*, monogyne *S. invicta*, and monogyne *S. richteri* workers. Results are interpreted with respect to population structure and potential lineage divergence between the two *S. invicta* social forms.

METHODS

Collection and Rearing of Laboratory Colonies

Groups of nearest-neighbor (<5-m) polygyne mounds were collected at least 10 weeks before testing from individual locales near the north-central Florida towns of Williston, Micanopy, and Ocala. Queens, workers, and brood were established in the laboratory without nest soil (Banks *et al.*, 1981). The Micanopy and Ocala locales are approximately 12 km apart, and both are 28 km from the Williston site. Mounds were considered functionally polygyne throughout testing based on small worker size (Greenberg *et al.*, 1985) and the presence of numerous, inseminated reproductives (verified by dissection). Each polygyne mound, referred to hereafter as a "colony," contained roughly equivalent numbers of workers and brood.

Monogyne *S. invicta* colonies were reared in the laboratory from newly mated queens (Banks *et al.*, 1981) collected in Gainesville, Florida, 8 months prior to tests. The Gainesville site is located 16, 24, and 40 km, respectively, from the Micanopy, Williston, and Ocala polygyne populations. Monogyne *S. richteri* colonies were collected in Lee County, Mississippi, 17 months prior to tests. These colonies were designated pure *S. richteri* based upon gas-liquid chromatographic (GC) analysis of venom constituents and cuticular hydrocarbons (Vander Meer *et al.*, 1985; Ross *et al.*, 1987a).

All colonies were placed in rearing trays (52.0 × 39.0 × 7.5 cm) and provided with petri dish nest cells (diameter, 14.0 cm) and cotton-stoppered water tubes (Obin, 1986). Colonies were reared exclusively on either thawed housefly pupae, hard-boiled egg, and honey-water (diet 1) or roaches (*Periplaneta americana*), moth pupae (*Anticarsia gemmatilis*), and honey-cane syrup solution (diet 2). Rearing temperatures were maintained between 21 and 27°C, and the light:dark cycle was variable.

Nestmate Recognition Bioassay and Data Analysis

The recognition bioassay (Obin, 1987) measured agonism in the context of nest defense. Individual workers designated "intruders" were allowed to walk onto a pair of forceps and were then introduced into rearing trays of "resident" polygyne colonies. Only ants that walked undisturbed from the forceps into the resident colony were tested. Intruders were positioned to maximize both the distance to any resident ants and the distance from any previous introduction. Observations were made from 15 cm away by an observer (M.O.) wearing a particle mask to minimize the agitation-inducing effects of exhalation on the ants. Individual intruders were removed from resident colonies with feather forceps immediately after testing. Replicate introductions into any one tray were spaced 15–20 min apart. Bioassays were conducted at 26–27°C.

Colony response to each intruder was scored on a (1–9) scale of increasing aggressive behavior (Table I). To avoid problems posed by potential nonindependence of behavioral acts, we recorded only the most aggressive response observed during an intruder's interaction with 20 resident ants. Resident colony responses for each treatment were computed from these individual introduction scores (Table II). Because assumptions of ANOVA could not be met (i.e., transformation-resistant heteroscedasticity), significantly different treatment pairs were identified by a nonparametric simultaneous test procedure based on the Mann–Whitney *U* statistic (Sokal and Rohlf, 1981; modified from Dwass, 1960).

Response to Intruders from Polygyne Colonies

These introductions measured the recognition response among polygyne colonies. To investigate the contribution of heritable cues to colony odor, tests were conducted among colonies reared on the same diet (diet 1). We first con-

Table I. Behavioral Units and Aggression Scores (1–9) Used to Assess Nestmate Recognition

Score	Behavior
9	Immediate lunge, grab, and stinging; intruder killed
8	Opponent surrounded and "held" in mandibles; appendages pulled/bitten off; eventual stinging; intruder killed
7	Intruder held (as in No. 8) but released; biting, abdomen-curling (stinging position), but no stinging
6	As in No. 7, but no abdomen-curling
5	Alarm (running, abdomen elevation, and vibration) and recruitment
4	Mandible gaping; rapid antennation; "sidling" (maintaining a lateral orientation to and slowly circling intruder)
3	Rapid antennation with antennae extended for more than 2 s
2	Antennation for less than 2 s. If mobile, intruder is followed slowly for several cm; if intruder stationary, worker stops
1	As in No. 2, but intruder does not induce following or stopping

Table II. Nestmate Recognition Scores Elicited from Laboratory-Reared Polygyne *S. invicta* (Si) Resident Colonies by Laboratory-Reared *S. invicta* or *S. richteri* (Sr) Intruders

Intruder type (<i>N</i>)	Treatment		Resident response ^a		
	Diet	Collection site	Mean $\pm$ SD*	Mode	Range
1. Nestmate control (21)	—	—	1.67 $\pm$ 0.58A	2	1-3
2. Polygyne Si (42)	Same	Same	3.98 $\pm$ 1.75B	3, 4	1-7
3. Polygyne Si (24)	Same	Different	3.25 $\pm$ 1.90B	3, 4	1-6
4. Polygyne Si (30)	Different	Different	6.70 $\pm$ 1.80D	8	2-9
5. Monogyne Si (21)	Same	Different	5.29 $\pm$ 1.42C	6	3-7
6. Monogyne Si (21)	Different	Different	8.00 $\pm$ 0.77E	8	6-9
7. Monogyne Sr (21)	Same	Different	8.79 $\pm$ 0.43F	9	8-9

^aResident colony response to each treatment was computed from individual introduction scores (Table I). Higher scores reflect greater aggression directed toward intruders.

*Scores followed by different letters are significantly different ($P < 0.05$) as determined by a nonparametric simultaneous test procedure based on the Mann-Whitney *U* statistic (see Methods).

ducted control introductions with seven Micanopy colonies by removing three workers from each colony and reintroducing them to another position in their original colony ($n = 21$). Using these same colonies, we next obtained one replicate of each possible intruder/resident colony pair ($N = 7 \times 6 = 42$) to measure within-population recognition. The between-population recognition response of six additional Micanopy mounds was measured against intruders from two polygyne mounds from each of the two other collection locales ($N = 6 \times 4 = 24$). The combined effects of locale and diet were measured 48 h later in these same six mounds by introducing intruders reared on diet 2 from each of two Williston mounds and three Ocala mounds ($N = 6 \times 5 = 30$).

Responses to Intruders from Monogyne Colonies

These introductions measured the recognition response of polygyne *S. invicta* residents reared on diet 1 toward monogyne *S. invicta* reared on either diet 1 or diet 2 and toward *S. richteri* intruders reared on diet 1. The effect of heritable recognition cues was assessed by testing seven Micanopy mounds (diet 1) against intruders from three *S. invicta* (diet 1) and three *S. richteri* (diet 1) colonies ($N_1 = N_2 = 7 \times 3 = 21$). The effect of environmentally acquired cues on recognition between social forms was tested by introducing workers from three monogyne *S. invicta* colonies reared on diet 2 ($N = 7 \times 3 = 21$). Each resident colony received one of the three types of intruders in a randomized sequence.

RESULTS

Response to Polygyne Intruders

Aggression scores elicited by intruders from polygyne colonies are presented in Table II. Behavior directed at nestmate controls (row 1; statistical mode = 2) was significantly less aggressive than behavior directed at nonnestmates reared on the same diet as resident colonies (row 2; mode = 4). When first introduced, intruders reared on the same diet as residents exhibited no detectable alarm or escape behavior and never initiated aggression upon contacting resident workers. Forty-five percent of nonnestmates (30 of 66) were attacked (elicited behavioral scores, >3), but no intruders were killed (elicited scores of 8 or 9). Scores generated by introductions between collection sites (row 3; mode = 3) were not significantly different from scores of within-site tests (row 2; mode = 3, 4). Behavior elicited by Williston intruders (mean $\pm$ SD = 3.17 ± 1.64 , $N = 12$) was not significantly different from behavior elicited by Ocala intruders (mean $\pm$ SD = 3.33 ± 1.61 , $N = 12$; $P > 0.05$, t test). Significantly more aggression was obtained in tests between colonies reared on different diets (row 4; mode = 8). Resident ants attacked 90% of all intruders (27 of 30), killing 50% of them. Although some intruders appeared to avoid contact with resident workers and exhibited alarm (retreat and rapid movement) after first contact, no intruders initiated aggression.

Response to Monogyne Conspecifics and Heterospecifics

Monogyne *S. invicta* intruders elicited significantly more aggression than polygyne intruders, both when residents and intruders were reared on the same diet (row 5; mode = 6; compare row 2) and when residents and intruders were reared on different diets (row 6; mode = 8; compare row 4). Interactions between residents and intruders reared on different diets were significantly more aggressive than interactions between residents and intruders maintained on the same diet. No intruders were killed when intruders and residents were reared on the same diet, but intruders were killed in 81% of between-diet introductions (17 of 21).

Although reared on the same diet as polygyne *S. invicta* colonies into which they were introduced, all *S. richteri* intruders were killed (row 7; mode = 9). The resulting aggression score was significantly greater than that obtained by either within-diet (row 5; mode = 6) or between-diet (row 6, mode = 8) introductions of monogyne *S. invicta*.

Interaction between monogyne *S. invicta* intruders and polygyne residents were distinguished by frequent aggression (mandible gaping, biting, and abdomen curling) initiated by the intruder (see also Morel *et al.*, 1990). However, intruder aggression did not significantly affect the magnitude of aggression elic-

ited from resident workers. For example, when residents and monogyne *S. invicta* intruders were reared on the same diet, response scores from introductions in which intruders initiated aggression (mean $\pm$ SD = 5.13 ± 1.25 , range = 3–6, $N = 8$) were not significantly different from scores of introductions in which intruders did not initiate aggression (mean $\pm$ SD = 5.38 ± 1.56 , range = 3–7, $N = 13$; $P > 0.05$, t test).

DISCUSSION

Environmental and Heritable Inputs to Colony Odor

Results suggest that, as in monogyne fire ants (Obin, 1986; Obin and Vander Meer, 1988) and other social insect species (Wilson, 1971; Breed and Bennett, 1987), both environmentally derived and relatively stable heritable cues (discriminators) can contribute to the colony odor of polygyne *S. invicta*. The potential adaptive significance of environmentally acquired components of colony odor in polygyne fire ants is indicated by the pronounced aggression (50% mortality) observed when intruders and residents were reared on different diets. These data support the contention (Stuart, 1987; Breed and Bennett, 1987) that recognition templates based on environmental inputs to colony odor have evolved under selection favoring colony autonomy and defense (rather than intranidal nepotism).

The contribution of heritable discriminators (either queen or worker derived) is suggested by the magnitude of aggression observed between polygyne non-nestmates maintained under uniform laboratory conditions. Among these colonies, between- and within-site recognition scores were not significantly different, suggesting that little population structure exists among the sites sampled in this study with respect to the genetic determinants of colony odor in polygyne *S. invicta* (cf. Stuart, 1987; Breed and Bennett, 1987). This inference is consistent with the extremely small genetic distances that characterize population pairs of fire ants in the southeastern United States (Ross *et al.*, 1987b).

It has been suggested (Brian, 1983; Ross *et al.*, 1987b) that alterations in ant social organization are associated with speciation and diversification. Heritable recognition cues (discriminators) may provide a sensitive measure of lineage differentiation, both because species identity is initially promoted by recognition systems (Wiley, 1981; Spiess, 1987) and because differences at only a single recognition locus can be dramatically expressed in behavior (Schofield *et al.*, 1982; Neigel and Avise, 1983; Yamazaki *et al.*, 1983). Although southeastern populations of the two *S. invicta* social forms have not been distinguishable at the level of protein polymorphism (Ross *et al.*, 1987b), the graded response of polygyne colonies to polygyne conspecifics, monogyne conspecifics, and monogyne heterospecifics reported here (Table II, rows 3, 5, and 7) supports

the notion of incipient speciation between the monogyne and the polygyne forms. However, the lower level of aggression directed toward polygyne intruders as a whole could also reflect greater discriminator variability in polygyne colonies and, thus, the increased likelihood of an individual intruder presenting an "acceptable" odor profile. This possibility is suggested by the fact that maximal responses to individual polygyne and monogyne intruders were similar, but the minimal responses elicited by polygyne intruders were lower (Table II; compare rows 3 and 5, 4 and 6).

Accounting for Heightened Nestmate Recognition in the Laboratory

Employing the same bioassay used in the present study, Morel *et al.* (1990) demonstrated that responses of freshly collected polygyne *S. invicta* to intruders of either social form was limited almost exclusively to antennation (behavioral scores 1–3). In contrast, polygyne workers in the present laboratory study were aggressive despite the presence of multiple queens and multiple matrilines. This result appears not to support the *discriminator variability* hypothesis. However, a threshold number of queens or matrilines may be required to generate colony odor templates sufficiently "wide" to reduce nestmate recognition to the level observed in natural colonies. By rearing mounds separately, we precluded the exchange of queens, workers, and brood that occurs within polydomous colonies (Glancey and Lofgren, 1988; other examples given by Breed and Bennett, 1987), thereby potentially reducing discriminator variability to levels commensurate with the formation of "narrow" colony odor templates. This explanation is particularly attractive, because it can be extended to accommodate environmentally derived cues that are also transferred among mounds during queen, worker and brood exchange.

The lack of nestmate recognition observed in the field could also be due to poorly formed as distinguished from "wide" worker templates. In fact, the *temporal* variability of colony odor cues that may typify polydomous societies could impede template formation altogether. First, it could alter immediate learning of the dynamic colony odor if such learning involved habituation. Second, temporal cue variability could "interfere" (Domjan and Burkhard, 1986) with postlearning memory consolidation in a manner similar to that which occurs when one cannot accurately recall a just-learned phone number after dialing another number. We may have facilitated the formation of defined recognition templates by rearing mounds separately, thereby promoting a more temporally stable cue milieu. The nature and effect of cue variability on template formation and empirical distinctions between wide and poorly formed templates remain challenges for future recognition studies.

Finally, differences in aggression may reflect differences in worker motivation between monogyne and polygyne colonies. The relative proportion of

queens, brood, and workers, undoubtedly altered when polygyne colonies were collected, may affect worker motivation for aggression. In addition, if the extent to which workers escalate encounters with nonnestmate conspecifics is positively correlated with resource quality and dependability or with the nutritional state of workers or queens, the heightened aggression observed between polygyne colonies in this study could reflect consistent *ad libitum* feeding in the laboratory. In conclusion, we suggest that elucidation of the factors responsible for the expression of nestmate recognition response in laboratory-reared polygyne *S. invicta* will illuminate ecological pressures and proximate mechanisms underlying the reduced intercolony discrimination observed in natural polygyne colonies of this and other ant species.

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REFERENCES

- Banks, W. A., Lofgren, C. S., Jouvenaz, D., Stringer, C. E., Bishop, P. M., Williams, D. F., Wojcik, D. P., and Glancey, B. M. (1981). Techniques for collecting, rearing and handling imported fire ants. *USDA, SEA-AATS-S 21*: 1-9.
- Breed, M. D., and Bennett, B. (1987). Kin recognition in highly social insects. In Fletcher, D. J. C., and Michener, C. D. (eds.), *Kin Recognition in Animals*, John Wiley, New York, pp. 243-286.
- Brian, M. W. (1983). *Social Insects*, Chapman & Hall, New York.
- Domjan, M., and Burkhard, B. (1986). *The Principles of Learning and Behavior*, Brooks/Cole, Monterey, CA.
- Dunton, R. F., Vinson, S. B., and Johnston, J. S. (1991). Unique isozyme electromorphs in polygynous red imported fire ant populations. *Biochem. Syst. Ecol.* **19**: 453-460.
- Dwass, M. (1960). Some k-sample rank-order tests. In Olkin, I. (ed.), *Contributions to Probability and Statistics, Essays in Honor of Harold Hotelling*, Stanford University Press, Stanford, CA, pp. 198-202.
- Gadagkar, R. (1985). Kin recognition in social insects—a review of recent findings and a consideration of their relevance for the theory of kin selection. *Proc. Indian Acad. Sci.* **94**: 587-621.
- Glancey, B. M., and Lofgren, C. S. (1988). Adoption of newly-mated queens: A mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. *Fla. Entomol.* **71**: 581-587.
- Glancey, B. M., Craig, C. H., Stringer, C. E., and Bishop, P. M. (1973). Multiple fertile queens in colonies of the imported fire ant, *Solenopsis invicta*. *J. Ga. Entomol. Soc.* **8**: 237-238.
- Greenberg, L., Fletcher, D. J. C., and Vinson, S. B. (1985). Differences in worker size and mound distribution in monogyne and polygynous colonies of the fire ant *Solenopsis invicta* Buren. *J. Kans. Entomol. Soc.* **58**: 9-18.
- Halliday, R. B. (1983). Social organization of meat ants *Iridomyrmex pupureus* analyzed by gel electrophoresis of enzymes. *Insectes Soc.* **30**: 45-56.

- Hölldobler, B., and Carlin, N. F. (1987). Anonymity and specificity in the chemical communication signals of social insects. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **161**: 567-581.
- Hölldobler, B., and Michener, C. D. (1980). Mechanisms of identification and discrimination in social Hymenoptera. In Markl, H. (ed.), *Evolution of Social Behavior: Hypotheses and Empirical Tests*, Verlag Chemie, Weinham, pp. 35-58.
- Hölldobler, B., and Wilson, E. O. (1977). The number of queens: An important trait in ant evolution. *Naturwissenschaften* **64**: 8-15.
- Holmes, W. G., and Sherman, P. W. (1982). The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* **27**: 491-517.
- Mabelis, A. A. (1979). Wood ant war: The relationship between aggression and predation in the red wood ant (*Formica polyctena* Forst.). *Neth. J. Zool.* **29**: 451-520.
- Mirenda, J. T., and Vinson, S. B. (1982). Single and multiple queen colonies of imported fire ants in Texas. *Southwest. Entomol.* **7**: 134-141.
- Hölldobler, B. and Wilson, E. O. (1977). The number of queens: An important trait in ant evolution. *Naturwissenschaften* **64**: 8-15.
- Holmes, W. G., and Sherman, P. W. (1982). The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* **27**: 491-517.
- Mabelis, A. A. (1979). Wood ant war: The relationship between aggression and predation in the red wood ant (*Formica polyctena* Forst.). *Neth. J. Zool.* **29**: 451-520.
- Mirenda, J. T., and Vinson, S. B. (1982). Single and multiple queen colonies of imported fire ants in Texas. *Southwest. Entomol.* **7**: 135-141.
- Morel, L., Vander Meer, R. K., and Lofgren, C. S. (1990). Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **83**: 642-647.
- Neigel, J. E., and Avise, J. C. (1983). Histocompatibility bioassays of population structure in marine sponges. Clonal structure in *Verongia longissima* and *Iotrochota birotulata*. *J. Hered.* **74**: 134-140.
- Obin, M. S. (1986). Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae): Effect of environment and the role of cuticular hydrocarbons. *J. Chem. Ecol.*, **12**: 1965-1975.
- Obin, M. S. (1987). *Experimental Analysis of Nestmate Recognition in the Imported Fire Ant Solenopsis invicta Buren (Hymenoptera: Formicidae)*, Ph.D. thesis, University of Florida, Gainesville.
- Obin, M. S., and Vander Meer, R. K. (1988). Sources of nestmate recognition cues in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Anim. Behav.* **36**: 1361-1370.
- Obin, M. S., and Vander Meer, R. K. (1989). Nestmate recognition in fire ants (*Solenopsis invicta* Buren): Do queens label workers? *Ethology* **80**: 255-264.
- Peterson-Braun, M. (1982). Intraspezifisches Aggressionsverhalten bei der Pharaonische *Monomorium pharaonis* L. (Hymenoptera, Formicidae). *Insectes Soc.* **29**: 25-33.
- Ross, K. G., and Fletcher, D. J. C. (1985). Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **17**: 349-356.
- Ross, K. G., Vander Meer, R. K., Fletcher, D. J. C., and Vargo, E. (1987a). Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution* **41**: 283-291.
- Ross, K. G., Vargo, E. L., and Fletcher, D. J. C. (1987b). Comparative biochemical genetics of three fire ant species in North America, with special reference to the two social forms of *Solenopsis invicta* (Hymenoptera: Formicidae). *Evolution* **41**: 979-990.
- Scofield, V. L., Schlumperger, J. M., West, L. A., and Weisman, I. L. (1982). Protochordate allorecognition is controlled by MHC-like gene system. *Nature* **285**: 499-502.
- Sokal, R. R., and Rohlf, F. J. (1981). *Biometry*, W. H. Freeman, San Francisco.
- Spies, E. B. (1987). Discrimination among prospective mates in *Drosophila*. In Fletcher, D. J. C., and Michener, C. D. (eds.), *Kin Recognition in Animals*, John Wiley, New York, pp. 75-119.
- Stuart, R. (1987). Transient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. *Behav. Ecol. Sociobiol.* **21**: 229-235.

- Traniello, J. F. A. (1982). Population structure and social organization in the primitive ant *Amblyopone pallipes* (Hymenoptera: Formicidae). *Psyche* **89**: 65-80.
- Vander Meer, R. K. (1988). Behavioral and biochemical variation in the fire ant *Solenopsis invicta*. In Jeanne, R. L. (ed.), *Interindividual Behavioral Variability in Social Insects*, Westview Press, Boulder, CO, pp. 223-255.
- Vander Meer, R. K., Lofgren, C. S. and Alvarez, F. M. (1985). Hybridization in fire ants: Biochemical and behavioral evidence. *Fla. Entomol.* **68**: 501-506.
- Wiley, E. O. (1981). *Phylogenetics: The Theory & Practice of Phylogenetic Systematics*, John Wiley, New York.
- Wilson, E. O. (1971). *The Insect Societies*, Belknap Press, Cambridge, MA.
- Yamazaki, K., Beauchamp, G. K., Egorov, I. K., Bard, J., Thomas, L., and Boyse, E. A. (1983). Sensory distinction between H-2b and H-2bm1 mutant mice. *Proc. Natl. Acad. Sci. USA* **80**: 5685-5688.