

Between- and Within-Species Recognition Among Imported Fire Ants and Their Hybrids (Hymenoptera: Formicidae): Application to Hybrid Zone Dynamics

MARTIN S. OBIN¹ AND ROBERT K. VANDER MEER²

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ABSTRACT Inter- and intraspecific recognition capability among laboratory colonies of *Solenopsis invicta* Buren, *Solenopsis richteri* Forel, and their hybrids were measured with aggression bioassays. No significant differences were detected when the ability of each population to discriminate intruders from the other two populations was compared. However, scores for within-population discrimination were highest among hybrids and lowest for *S. invicta*, suggesting greater genetic variability for heritable recognition cues ("discriminators") among hybrids and least variability in *S. invicta*. The results support the hypothesis that the fire ant hybrid zone is maintained in part by hybrid superiority resulting from increased genetic variability in heterozygotes. The hierarchy of genetic variability suggested by the data is contrasted with that suggested by electrophoretic studies of "neutral" Mendelian markers in fire ant populations.

KEY WORDS Insecta, *Solenopsis*, nestmate recognition, hybrids

WITH THE DOCUMENTATION of introgression (hybridization) between the red imported fire ant, *Solenopsis invicta* Buren, and the black imported fire ant, *S. richteri* Forel (Vander Meer et al. 1985, Vander Meer 1986), the imported fire ant has emerged as an important model system for systematics and biogeography (Ross et al. 1987a,b; see also Buren et al. 1974). Hybridization appears extensive throughout Mississippi, Alabama, and northwestern Georgia (Ross et al. 1987a, Vander Meer & Lofgren 1988) and has probably been occurring since contact was first established in southern Alabama in the 1940s (Wilson 1958, Vander Meer & Lofgren 1988). However, only the Mississippi hybrid zone is parapatric with both parental types (Vander Meer & Lofgren 1988; see also Lofgren 1986). Current interest surrounds postulated mechanisms that explain the temporal stability and northward extension of hybrid fire ant populations and the current restriction of *S. richteri* to a small region of northeastern Mississippi (Ross et al. 1987a, Vander Meer & Lofgren 1988).

Several explanations have been advanced for the geographic and historical dynamics of the fire ant hybrid zone in east-central Mississippi (Ross et al. 1987a). The most plausible invoke either hybrid superiority in habitats "intermediate" to those favoring parental forms ("bounded hybrid superiority" of Moore [1977]), and perhaps resulting from increased genetic diversity in recombinants; a "wave of advance" (Barton & Hewitt 1985) of the com-

petitively superior *S. invicta*; or a balance between dispersal and selection against hybrids ("tension zone" model of Barton & Hewitt [1985]). Little is known concerning the applicability of these models to the biogeography of imported fire ants. However, Ross et al. (1987a,b) recently demonstrated low levels of genetic variability in the parental forms and obtained significantly higher heterozygosity values in hybrids. These data are consistent with a "hybrid superiority" mechanism.

In this study, we use intraspecific (nestmate) and interspecific (species) recognition as a measure of relative competitive ability and genetic diversity in the fire ants *S. invicta* and *S. richteri* (sensu Buren 1972) and their Mississippi hybrids. Intra- and interspecific competitive ability is an essential determinant of colony fitness and ant community structure (Hölldobler & Lumsden 1980, Levings & Traniello 1981, Hölldobler 1983). This competition is mediated to a significant degree by discrimination of chemical cues for territory, trail, and competitor-enemy recognition (reviewed in Hölldobler & Carlin 1987). Fire ants maintain colony-specific territories and exhibit a complex alarm-recruitment defense behavior when intruders are encountered in or near the nest (Wilson 1962, Obin & Vander Meer 1985, Obin 1987). Intruders are recognized by olfactory cues that distinguish them from colony members. These chemical "labels" may be heritable ("discriminators" of Hölldobler & Michener [1980]) or environmentally acquired (Obin 1986, 1987, unpublished data for *S. richteri*; Obin & Vander Meer 1988). Here we use an aggression bioassay in the laboratory to assess the ability of the two parental fire ant species and their hybrid to detect conspecific and congeneric "intruders."

¹ Department of Zoology, University of Florida, Gainesville, Fla. 32611. Current address: Division of Pathology Research, Brigham & Women's Hospital, 75 Francis Street, Boston, Mass. 02115.

² USDA-ARS, P.O. Box 14565, Gainesville, Fla. 32604.

Table 1. Recognition response of *S. invicta* (Si), *S. richteri* (Sr), and *S. invicta* × *S. richteri* hybrids (HY) maintained in the laboratory

Set no.	Int × Res	(n)	Aggression level (%/n)		
			I	II	III
1	Sr × Si	(50)	0.0/0	0.0/0	100.0/50a
2	HY × Si	(25)	0.0/0	0.0/0	100.0/25a
3	Si × Si	(72)	8.3/6	58.3/42	33.3/24c
4	Si × Sr	(50)	0.0/0	0.0/0	100.0/50a
5	HY × Sr	(25)	0.0/0	0.0/0	100.0/50a
6	Sr × Sr	(72)	4.2/3	36.1/26	59.7/43b
7	Si × HY	(25)	0.0/0	0.0/0	100.0/25a
8	Sr × HY	(25)	0.0/0	12.0/3	88.0/22a
9	HY × HY	(20)	0.0/0	0.0/0	100.0/25a

Within-group tests measured intraspecific (nestmate) recognition, whereas between group tests measured interspecific (species) recognition. Colony response to each intruder ant (Int) was scored as the highest level (I–III) of aggression directed at that intruder by 20 resident ants (Res). The frequency distributions of Level I, II, and III behaviors were tested for homogeneity by contingency tables, using the G statistic or by Fisher exact tests. Inspection of the data determined which comparisons were required to construct maximal, nonsignificant sets of distributions. The following sets of distributions were compared: 3 versus 6, 4 versus 6, 8 versus 6, and 7 versus 8. Distributions of behavior followed by different letters are considered significantly different ($\alpha = 0.01$).

By controlling for differences in environmentally derived recognition cues, we also were able to evaluate indirectly the relative diversity of heritable recognition cues in the three populations studied. These data are compared with heterozygosity values obtained from isozyme data (Ross et al. 1987b) and interpreted with respect to temporal and geographic dynamics of the Mississippi fire ant hybrid zone.

Materials and Methods

Collection and Rearing of Stock Colonies. *Solenopsis invicta* were reared from newly mated queens (Banks et al. 1981) collected in Alachua County, Fla., 17–23 mo before tests. *S. richteri* and hybrid colonies were collected 12 mo before tests from single Mississippi locales (populations), situated about 500 mi from the *S. invicta* collection sites. The *S. richteri* were collected at mile 279 on the Natchez Trace in Lee County. The hybrids were collected about 30 mi away in Loundes County, 14 mi west of Columbus on Interstate 82. The queen and several thousand workers and brood were collected from each colony.

Each colony was determined to be *S. invicta*, *S. richteri*, or hybrid by gas-liquid chromatographic (GC) analysis of pooled samples of worker cuticular hydrocarbons and venom alkaloids (Vander Meer et al. 1985, Ross et al. 1987a). This method is highly concordant with population designations based on enzyme polymorphism. A numerical index was developed with the GC data to identify hybrid and parental populations (Ross et al. 1987a). Hybrid colonies with "hybrid indices" closest to 0.5 (the

midrange between values for the parental species) were selected for the present study.

Colonies were maintained in Petri dish nests placed in trays (52.0 by 39.0 by 7.5 cm) that served as foraging arenas and were fed fly pupae (*Musca domestica* L.), hard-boiled egg, and honey-water. The photoperiod was variable.

Voucher specimens have been deposited at the Florida Division of Plant Industry.

Recognition Bioassay. The recognition bioassay measured agonism as nest defense against non-nestmate "intruders." Individual intruder ants walked undisturbed onto a pair of extended forceps and were then introduced into a "resident" colony. Intruders were positioned to maximize the initial distance between the site of introduction and resident ants as well as the distance from any previous introduction. Observations were made from a distance of 15 cm, and the observer (M.O.) wore a particle mask to minimize the agitation-inducing effects of exhalation on the ants. Intruders were tested once and were removed from resident colonies with feather forceps after they had encountered 20 ants. Ten colonies each of *S. invicta* and *S. richteri* and five hybrid colonies were used during the experiments. All nine possible reciprocal combinations of between- and within-group (population) introductions were conducted (Table 1). Interspecific tests were performed with five randomly selected colonies of each parental form (residents) and intruders from ten colonies of the other parental form; each resident colony was tested against one intruder from each intruder colony ($n_1 = n_2 = 50$). This was done so that half the colonies of each parental species would be naive with respect to chemical recognition cues possessed by heterospecific intruders and potentially shared with the hybrids. The five naive colonies of each parental species were tested against individual intruders from the five hybrid colonies ($n_3 = n_4 = 25$). Hybrid colonies were tested against intruders from five randomly selected colonies of each of the parental forms ($n_5 = n_6 = 25$).

Intraspecific tests were performed with nine colonies of each of the parental forms (residents) tested against individual intruders from the other eight conspecific colonies ($n_7 = n_8 = 72$). Each of the five hybrid colonies was tested against intruders from the other four ($n_9 = 20$). Bioassays were conducted at 23–24°C.

Data Analysis. Resident ant responses to each intruder were scored as follows:

Level I—Investigation: Intruder antennated; if mobile, intruder may or may not be followed for several centimeters; if intruder is stationary, resident ant may or may not stop; rapid antennation of intruder ("drumming").

Level II—Challenge—defend: Mandible gaping; repeated, rapid antennation; "sidling" (maintaining a lateral orientation to and slowly circling intruder); alarm (running, gaster elevation, and vibration) and recruitment; intruder held by petiole

in resident's mandibles but released; biting; abdomen-curling (stinging posture) by residents but no stinging.

Level III—Lethal attack: Intruder surrounded and held in residents' mandibles by petiole and appendages, and appendages are pulled or bitten off accompanied by eventual stinging; immediate lunge, grab, and stinging.

As a precaution against possible nonindependence of behavioral units, colony response to individual intruders was designated as the single most aggressive response directed toward the intruder by 20 resident ants. The null hypothesis of independence between populations confronting each other in the bioassay and the distributions of Levels I, II, and III behavior was tested by G tests (Sokal & Rohlf 1981). When no Level I behavior was recorded for an intruder \times resident type, frequencies of Level I and II behavior were "lumped", and degrees of freedom were adjusted accordingly. Fisher exact tests were performed when only Level III behavior was recorded for an intruder \times resident combination. Inspection of the data determined which comparisons were required to construct maximal, nonsignificant sets of distributions. Because four pairs of distributions were tested (Table 1), we set the comparison-wise "alpha" for rejection of the null hypothesis at 0.01.

Results

Data for between-population tests (Table 1) indicate that hybrid colony response to *S. invicta* and *S. richteri* was not significantly different from the response of either parental species to hybrids or heterospecifics. Similarly, hybrid response to hybrid intruders did not significantly differ from hybrid response to either of the parental species. In contrast, both parental species were significantly less aggressive toward conspecific intruders than toward heterospecific or hybrid intruders, and intraspecific recognition scores of *S. invicta* were significantly lower than scores of *S. richteri*.

Discussion

The lack of significant differences in aggressive responses of all six between-population sequences argues against either superior or inferior species-level recognition capabilities of hybrids. To the extent that this capability contributes to colony fitness (Hölldobler & Carlin 1987), these results fail to support the "dynamic equilibrium" model (Barton & Hewitt 1985) as an explanation for the temporal stability of the Mississippi fire ant hybrid zone (see Ross et al. 1987a). In this model, the hybrid zone persists despite selection against hybrids as a consequence of gene flow (dispersal) from the parental populations.

Recognition assays have elucidated phylogenetic relationships of social insects (Le Moli et al. 1984) and population structure of clonal marine organ-

isms (Neigel & Avise 1983a,b), and we propose that such an approach can be extended to address the question of within-species genetic diversity. In our study, the intraspecific recognition response of each population was significantly less than its interspecific (species) recognition response. In contrast, recognition response among hybrid colonies did not significantly differ from hybrid response to either of the parental genotypes. Because recognition under the homogeneous laboratory conditions maintained in this study is theoretically based solely on heritable odor differences, these results are consistent with the hypothesis that genetic variability underlying the expression of heritable recognition cues (discriminators) is greater in hybrid populations than in populations of either parental species. This interpretation supports the isozyme data of Ross et al. (1987b), and the suggestion (Ross et al. 1987a) that stability of fire ant hybrid zones may be a consequence of enhanced genetic diversity and consequent hybrid superiority (see also Ross & Fletcher 1985, 1986). We propose a direct causal relationship between variability at "discriminator loci" and hybrid superiority, for discriminator expression is believed to reflect the contributions of polymorphic loci under strong selection by pathogens and parasites (Hepper 1986; Crozier 1986, 1987).

However, our behavioral data are not entirely concordant with the biochemical data of Ross et al. (1987b). Using presumably neutral Mendelian markers, they found heterozygosity values for *S. richteri* to be significantly lower than those for *S. invicta* sampled extensively throughout its range (Ross et al. 1987a). Their results are intuitively satisfying given the extremely small effective population size in *S. richteri* (Ross et al. 1987b). In contrast, behavioral data from the three populations sampled in this study suggest the following hierarchy of heterozygosity of loci on which selection is presumably acting: *S. invicta* < *S. richteri* < hybrids. To the extent that genetic diversity is reflected in colony fitness, our data do not support a "wave of advance" model (Barton & Hewitt 1985), in which the northward movement of the hybrid zone toward *S. richteri* populations is in the direction of the competitively inferior parental species.

In conclusion, the production of viable hybrids between bottlenecked populations may be an important mechanism of recombination in social Hymenoptera, a group distinguished by low levels of genetic variability (reviews in Graur 1985, Sheppard & Heydon 1986, Woods & Guttman 1987). This intriguing possibility should be addressed by extensive, long-term studies of natural fire ant populations here and in South America.

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