annulate part of antennal flagellum black. The frons is considerably narrower and less convergent below, the annulate portion of the antennal flagellum is relatively shorter, the basal plate broader, and the dorsal angle markedly more acute. The smoky wing apex of *woodruffi* also separates it from *brodzinskyi*. These character states can best be appreciated by reference to the accompanying figures of both species, drawn by the senior author with the aid of a camera lucida on a Wild binocular microscope, while the amber pieces were immersed in colorless mineral oil.

We take great pleasure in dedicating this important paleontological find to Dr. Robert E. Woodruff, who not only recognized the importance of this fossil and enable us to study it, but declined co-authorship. He also read and made astute suggestions on the several drafts of this paper.

COMMENTS

The discovery of two distinct but obviously similar species of tabanids in amber believed to be at least 25 million years old is an extraordinary occurrence considering the rarity of fossil Tabanidae. That these specimens show no character states that would separate them generically from a group of living sympatric and mainly precinctive Antillean species suggest that evolution in this group of Diptera has been much slower than in those groups of animals, such as horses, elephants, and primates, whose differentiation is believed to have occurred during the geologically abrupt changes of the Pleistocene.

ENDNOTE

Contribution No. 717, Bureau of Entomology, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32602.

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POLYGYNY IN HYBRID IMPORTED FIRE ANTS

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ABSTRACT

The occurrence of polygyny in hybrid fire ants from Mississippi was initially indicated by the clustering behavior of the workers around queens. Polygyny was demonstrated by the rate of oviposition of isolated queens, and dissection of samples of queens for the presence of sperm in the spermatheca. The colonies were identified as *S. invicta*/*S. richteri* hybrids by gas chromatograph analyses of venom alkaloids and cuticular hydrocarbons.

RESUMEN

La ocurrencia de hormigas de fuego híbridas polígamas de Mississippi fue inicialmente indicado por el comportamiento de agregación de los obreros alrededor de las reinas. Se demostró poligamia por la tasa de oviposición de reinas aisladas, y por la disección de muestras de reinas para determinar la presencia de esperma en la espermatocica. Se identificaron las colonias como híbridos de *S. invicta*/*S. richteri* usando análisis cromatográfico de gas de los alcaloides del veneno e hidrocarburos cuticulares.

The imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, are agricultural pests that affect several important crops. In addition, high population densities and the very aggressive nature of the fire ants pose well documented health hazards to some humans and animals occupying the same habitat (reviewed by Adams, 1986). The ants are found in 10 southeastern states and Puerto Rico and infest about 93,120,000 ha (Lofgren 1986).

Within the past 15 years, two important discoveries have been made by researchers working with the imported fire ants. The first discovery was polygyny (multiple functional queens) in *S. invicta* populations in Mississippi (Glancey et al. 1973). Since this initial report, polygyny has been reported from *S. invicta* colonies in Texas, Louisiana, Georgia, Florida, Oklahoma, Arkansas, and Alabama (Hung et al. 1974, Fletcher 1983, Lofgren & Williams 1984, Banks & Wojcik unpublished). Polygyny is known to occur in other species of *Solenopsis* in North and South America (Banks et al. 1973, Summerlin 1976, Jouvenaz et al. in press).

The second important discovery was the occurrence of viable hybrids of *S. invicta* and *S. richteri* (Vander Meer et al. 1985, Ross et al. 1987a). The hybridization phenomenon was first detected through biochemical characters (Vander Meer et al. 1985), with subsequent recognition of morphological characters (J. C. Trager unpublished). Subsequent studies by Diffie et al. (1988) and Vander Meer and Lofgren (unpublished) have shown that the hybrid has an extensive range in northeastern Mississippi, northern Alabama, and northwestern Georgia. The expanse of territory occupied by the hybrid as well as its reproductive viability (Ross et al. 1987a) have raised concerns about the possibility of range expansion of hybrid fire ants.

We report here evidence for polygynous hybrids of *S. invicta* and *S. richteri* in Mississippi, a unique situation which could further complicate the control of the imported fire ants in the United States.

MATERIALS AND METHODS

While collecting imported fire ants in northern Mississippi for studies requiring queen-rite colonies, BMG and DPW found what appeared to be multiple queen colonies. To collect a queen from a mound, a shovel-full of tumulus containing ants from the mound was scattered on the pavement. Any dealates which evoked a clustering behavior (a response to queen pheromones) were suspected of being mated (Glancey et al. 1975). When suspected polygynous colonies were recognized, the dealates were collected and placed in vials, and a sample of the colony was shoveled into a quart jar or 5 gal buckets. The buckets were lined with Fluon^{R} (Banks et al. 1981) to prevent escape of the ants. We found what appeard to be polygynous colonies at 3 separate locations. Each colony contained 2 or more dealated females. At the first location, on the shoulders of the frontage road, 1/2 mi east of US 82 and Alt US 45 junction, Lowndes Co. (ca. 14 miles W of Columbus), we collected 19 mounds of which 4 had several dealated females. The 4 mounds were within a 100 ft stretch of road. At the second location, on US 45, 1/2 mi south of Lauderdale (Lauderdale Co.), we collected 2 colonies, both with several deal-

ates. At the third location, 1/4 mi south of location #2, we collected 6 colonies of which one had several dealates.

To test whether or not these 7 colonies were indeed polygynous, dealates were collected and subjected to a 5 hr quantitative oviposition bioassay (Fletcher et al. 1980). After the oviposition bioassay, 11 of the queens from one nest, 7 from another nest, and 4 from a third nest were dissected and a spermathecal examination made.

The hybrid status of these colonies was verified using the methods of Vander Meer et al. (1985) and is briefly summarized here. Pooled samples of worker ants (50-100) from each colony were placed in a vial and soaked in hexane (HPLC grade, Burdick and Jackson, Muskegon, MI) for ca. 24 hr. After soaking, the solvent was transferred to a clean vial and saved for chemical analysis. Ethanol (70%) was added to the vial containing the ants to preserve a taxonomic sample. The hexane solution was analyzed for species-specific venom alkaloids and hydrocarbons by gas chromatography on a Varian^R 3700 gas chromatograph equipped with a flame ionization detector. A 30 m DB-1 fused silica capillary column was used to separate the components of interest. The temperature program (Ross et al. 1987a) was 150° to 285° C at 5 min. with a final temperature hold of 3 min.

Results and Discussion

All of the isolated dealates laid sufficient eggs in the oviposition bioassay to meet the criteria of Fletcher et al. (1980) for polygyny. All of the dissected queens contained sperm and were mated. The GC patterns of both the venom alkaloids and hydrocarbons showed that 26 of 27 collected colonies were hybrid colonies (Figure 1). The one exception was a polygynous *S. invicta* colony (from site 2).

This finding of hybrid polygynous imported fire ant colonies is interesting for several reasons. First, the occurrence of these colonies demonstrates some of the variability in the hybrid. Of the 26 hybrid colonies collected at these three locations, 20 colonies were apparently monogynous. Secondly, the finding of hybrid polygynous colonies, coupled with the widely scattered occurrence of polygyny throughout the range of *S. invicta* in North America, suggests that polygyny in *S. invicta* in North America has not arisen de novo as postulated by Ross et al. (1987b), but is in fact part of the *S. invicta* genome which is being expressed in North America. Polygyny in *S. invicta* has not been documented in South America, but we assume it occurs because polygyny has been observed in other *Solenopsis* spp. in South America (Jouvenaz et al. in press, Wojcik, Lofgren, & Jouvenaz unpublished). A generally accepted assumption is that the original introduction of the imported fire ants into North America occurred with only a few queens for each species (Tschinkel & Nierenberg 1983) which would limit the original North American gene pool. The presence of diploid males in monogynous and polygynous colonies is evidence that inbreeding is taking place in *S. invicta* (Ross & Fletcher 1985). This inbreeding may be the mechanism by which the polygynous phenotype is being expressed. One year after our initial discovery, we returned to the original collection area, collected samples, and found that the hybrid multiple queen colonies were still present.

The occurrence of polygynous *S. invicta* and *S. invicta/S. richteri* hybrid colonies could pose problems in efforts to control these fire ants. Current research is being directed toward the formulation or discovery of more species-specific control methods. These efforts have centered on the use of pheromones and the search for specific pathogens from their native homelands in South America. Our recent discoveries raise concern whether a species-specific pathogen of *S. invicta* will also be effective against the hybrid. In addition, would a toxic bait formulated with the species-specific pheromones of *S. invicta* be effective for control of the hybrid? Control of the polygynous pest ant *Monomorium pharaonis* with bait toxicants is difficult and inconsistent (Newton &

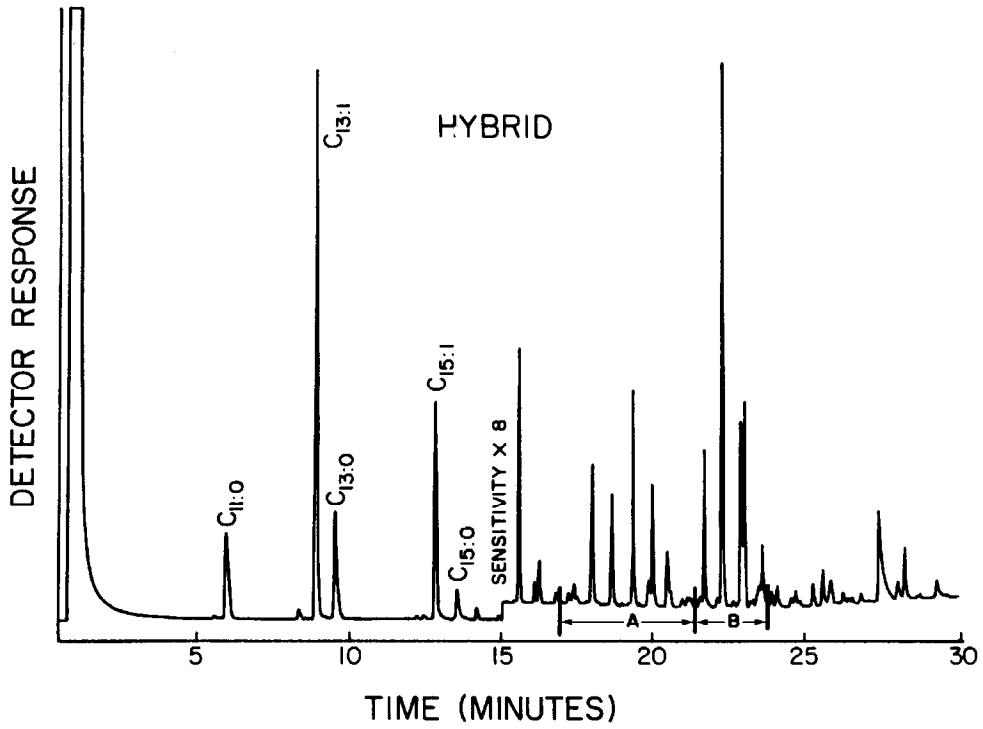

Fig. 1. Gas chromatogram of a hexane soak of hybrid worker ants. Venom alkaloids are designated by the chain length and the double bond status of the 6- alkyl or alkenyl group of the piperidine alkaloids associated with *S. richteri* and *S. invicta* (i.e. $C_{13:1}$ is 2-methyl tridecenyl piperidine). *S. richteri* associated hydrocarbons are defined by the components above A, and those associated with *S. invicta* are defined by the components above B. See Vander Meer et al. (1985) and Ross et al. (1987) for detailed comparison of the hybrid and parent chemistry.

Coombes 1987). A recent report (Glancey et al. 1987) indicates that control of polygynous *S. invicta* populations with Amdro^{R} bait has been less effective than that of monogynous populations. The rapid changes in this insect's ability to survive through polygyny and hybridization warrants continous investigation into its strategies for survival and efforts for its control.

Endnote

This article represents the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

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