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10 January 2009

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Dear Board Members,

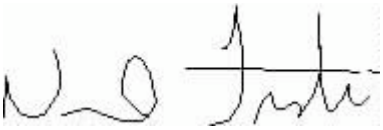
Since my research proposal was funded, we have completed testing the three hypotheses of the proposed research. We have published one paper (Torres et al. 2007, which I attached to a previous progress report), have a second paper *in press* (to be published in early 2009), and will submit a third paper within the next few weeks a third paper (to the prestigous journal PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES). We are extremely excited about our discoveries (described below), and I believe that this research has substantially advanced our understanding of both ant biology generally, and of Argentine ant sociality and chemical ecology in particular. We anticipate submission of at least one other manuscript that includes data collected during execution of this project. Below, I enumerate each of the proposed hypotheses and describe our findings for each.

I have also presented this research to a broad array of audiences. I have presented invited departmental seminars at several universities, including Ohio State University, University of Illinois, Scripps Institute of Oceanography, University of Miami, North Carolina State University, UCLA, UC-Davis, UC-Merced and UC-Berkeley. I have also presented our results at a number of scientific meetings including the annual meeting of the Entomology Society of America (twice) and the International Union for the Study of Social Insects.

Finally, we presented some of our preliminary data at the American Chemical Society meeting, in September 2006. The response to our work was overwhelmingly positive, and articles were written in several major publications, including high-profile features in THE LOS ANGELES TIMES (15 Sept. 2006; p. B1), THE SAN FRANCISCO CHRONICLE (15 Sept. 2006, p. B1), SCIENCE NEWS (2006; vol. 170, p. 222), NATIONAL GEOGRAPHIC NEWS (22 Sept. 2006), and NATIONAL PUBLIC RADIO (All Things Considered; 15 Sept. 2006).

Thank you, again, for your support of this important research.

Sincerely,



Neil Tsutsui

Final Report

Hypothesis 1. The genetic diversity and hydrocarbon diversity of colonies are positively correlated.
Progress: This work has been completed. The paper that we have written describing our results is, by far, the most comprehensive analysis of genetics and chemical ecology that has ever been performed for the Argentine ant. This paper is currently *in press* at the highly-regarded journal, MOLECULAR ECOLOGY and will be published in early 2009. I briefly describe below our research and findings.

To test the relationship between genetic diversity and hydrocarbon diversity in supercolonies of Argentine ants, we collected ants from 25 locations distributed across four different continents. We extracted DNA from several hundred ants and performed DNA fingerprinting using nuclear microsatellite loci. We analyzed the chemical profiles on the exoskeletons of these ants (the exact same individuals as used for the DNA fingerprinting) using gas chromatography and mass spectroscopy (GC/MS). This research has revealed several important findings: 1) We show that a significant genetic bottleneck occurred during introduction of the Argentine ant to all parts of its introduced range (including Europe, which had been debated in previous publications), 2) We show a concomitant reduction in cuticular hydrocarbon (CHC) diversity in introduced populations relative to native populations, and 3) We statistically demonstrate the positive correlation between genetic diversity and hydrocarbon diversity.

Hypothesis 2. Application of hydrocarbons from one colony to workers in a behaviorally different colony will trigger aggressive rejection of the treated worker by her nestmates.
Progress: This work has been completed and was published in the journal INSECTES SOCIAUX in 2007 (Torres et al. 2007). I have attached a copy of the paper, and review the research and findings in the following paragraph.

In this study, we extracted CHCs from workers in all known California supercolonies, and applied them to the exoskeletons of individual, living workers from foreign colonies. We demonstrated that this treatment causes aggressive rejection of workers by their nestmates, as they are mistaken for members of foreign Argentine ant colonies. We have also shown that this aggressive response is concentration-dependant, and used GC-MS to analyze the specific CHC changes that occur.

Hypothesis 3. Pure (synthesized) versions of the hydrocarbons that vary most among colonies, but do not vary within colonies, can be used to trigger aggression when applied to individual ants.
Progress: We have completed this research, and have written a draft of the first paper that describes our results. This is truly groundbreaking work, as this is the first study to identify the specific chemicals that ants use to recognize nestmates, synthesize them in pure form, and demonstrate that these pure versions alter the behavior of living ants. We will submit this paper to the journal PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES by the end of January 2009. This work has already received significant media attention (see letter above).

There are four main conclusions of this research: 1) Treatment with our synthetic hydrocarbons is sufficient to trigger aggression among normally cooperative nestmats, 2) Different colonies display different reactions to the same hydrocarbon, 3) The level of aggression displayed is proportional to the amount of synthetic hydrocarbon that is applied, and 4) Combinations of different hydrocarbons trigger higher levels of aggression than individual hydrocarbons.