Proceedings of the Seventh International Conference on Urban Pests
William H Robinson and Ana Eugênia de Carvalho Campos (editors) 2011
Printed by Instituto Biológico, São Paulo, SP. Brazil.

HOW GENETIC STUDIES CAN HELP WITH SUBTERRANEAN TERMITE MANAGEMENT

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Abstract Here I review the application of genetic techniques to subterranean termite studies, focusing on their contributions to applied science. Current use of molecular genetic methods in the area of termite management fall into two main categories: 1.) taxonomy and species identification; and 2.) colony identification. DNA sequencing data are helping to clarify the poorly resolved state of subterranean termite taxonomy. These studies provide new tools for accurate species identification, helping to detect the presence of exotic species outside their native range and determine their geographic sources. Colony identification through DNA fingerprinting distinguishes large numbers of colonies and tracks them over extended periods. Such studies are providing information on the abundance of colonies in locations, the size and shape of foraging areas, and colony breeding structure. In addition, genetic methods allow for the evaluation of colony-level effects of termiticides applied in the field. This information has a role in the registration of new termiticides. The availability of a termite genome should provide many new promising targets for termite control, including genes critical to termites or their gut symbionts, that could be for silenced using RNAi or other methods.
Key Words Microsatellites, DNA, population genetics, pesticide efficacy

INTRODUCTION

Genetic tools have revolutionized biology, providing powerful methods to investigate nearly all levels of biological organization from the mechanisms of gene expression at the subcellular level to ecosystem function. Although termite biologists and other urban entomologists have been slow in adopting these methods, genetic techniques are now being used with increasing frequency in termite biology, and we are now beginning to reap the rewards. Much of this work has focused on the large and important group of subterranean termites (Rhinotermitidae).

Subterranean termites are widespread throughout the tropical and temperate regions of the world, where many species readily attacking human structures often causing severe damage. Owing to their cryptic, underground nesting and foraging habits, many features of their biology have been difficult to study using more traditional methods. The application of population genetic markers, primarily microsatellites, has shed much needed light on the breeding structure of colonies (number of reproductives and degree of relatedness among them), the size of colony foraging areas, and population dynamics. In addition, molecular genetic techniques have provided important new insights into the process of caste differentiation in this group. DNA sequence data have greatly improved our understanding of the taxonomy and invasion biology of subterranean termites. Here, I review the contributions of genetic methods to research efforts related to subterranean termite management. Vargo and Husseneder (2009) provide a more comprehensive review of the insights provided by genetic tools to subterranean termite biology and management.

Taxonomy and Species Identification

One of the most important steps in the management of any urban pest is proper species identification. However, this can be quite challenging in the case of subterranean termites. Species in this group are notoriously difficult to identify, with few distinguishing traits, especially among the workers and soldiers, the most commonly encountered castes. Thus it is not surprising that the taxonomy of this group is far from resolved. The recent application of molecular genetic data, sometimes in combination with morphological characters, cuticular hydrocarbon data, and flight phenologies, has helped to clarify subterranean termite taxonomy. A good example is the species of *Reticulitermes* in the U.S., where up until 5 or 6 years ago there were six recognized species, whereas now are there seven, due to the sinking of one species (*R. arenincola* Goellner = *R. flavipes* (Kollar)) (Austin et al., 2005) and the recognition of two new species, *R. okanaganensis* Szalanski et al. (Szalanski et al., 2006) and *R. malletei* Banks (Austin et al., 2007). These changes have occurred based primarily on molecular

data. Despite the recent advances in subterranean species taxonomy, many more studies of *Reticulitermes* and *Coptotermes* are needed, as these two genera account for about half of all the described subterranean species, most of which were described only in the last 70 years in China (Eggleton, 1999).

An important contribution of molecular methods has been the clarification of the taxonomic status of some invasive subterranean termites. For example, the termite previously known as *R. santonensis* Feytaud occurs in France, Hamburg, Germany and Chile, among other locations around the world. It was thought to be native to France but based on mtDNA sequence data has been shown to be *R. flavipes* from the U.S. (Clément et al., 2001; Uva et al., 2004). And the invasive species *Coptotermes gestroi* (Wasmann) was previously considered several different species, including *C. vastator* Light and *C. havilandi* Holmgren, which have now been synonymized (Kirton and Brown, 2003; Yeap et al., 2007).

Molecular studies of DNA sequence variation within and among species have yielded very useful PCR-based methods for rapid and accurate species determination for *Reticulitermes* spp. in the central and eastern U.S. (Szalanski et al., 2003; Foster et al., 2004) and for distinguishing *C. formosanus* Shiraki from other species of *Coptotermes* (Szalanski et al., 2004). These methods have distinct advantages over morphological keys: they give unambiguous results, they can be used with any caste or developmental stage, and they can be performed on a single individual. The application of these methods has allowed for more accurate determination of species ranges, detection of species outside their native areas, and determination of the relative abundance of species in particular geographic areas (reviewed in Vargo and Husseneder, 2009), all of which have important implications for subterranean termite management. Expanded use of rapid methods of species identification should prove immensely useful for improving our knowledge of the distributions of subterranean termite species.

Colony Determination

Subterranean termites are social insects that live in colonies, yet we still have a poor understanding of such basic features as the number and relatedness among breeders within colonies, the foraging ranges of colony, and the dynamics of colony-colony interactions. This is because the cryptic nature of the nesting and foraging habits of these insects make it difficult to locate reproductives within colonies and to determine the colony identity of foragers. The application of molecular genetic markers, especially microsatellites, has opened up a wealth of possibilities by permitting 'fingerprinting' of colonies to infer their breeding structure, to determine their foraging areas, estimate population densities, and to track the fate of colonies after insecticide treatment.

In addition to advancing our understanding the basic biology of subterranean termites, the application of genetic tools to identify colonies has made important contributions to termite management. For the past 20 or more years, marking termites using fat soluble dyes has been the method of choice for identifying individual colonies in the field and delimiting colony foraging areas (e.g., Su and Scheffrahn, 1988). While this technique has been a useful research tool, it has a number of limitations that make it difficult to study large numbers of colonies in the field, or study colonies over an extended period of time. These drawbacks are discussed by Parman and Vargo (2008), but briefly, the disadvantages are: 1.) large numbers of termites are required to effectively mark a colony; 2.) the process of installing monitoring stations, collect, mark, and recollect termites can be time consuming and labor intensive; 3.) there are a limited number of dyes available; and 4.) the dyes are short lived so that colonies do not retain dyes for extended periods. All of these limitations are overcome with genetic markers: even a few workers can be accurately identified to colony, only a single visit is required to a site to collect samples, genetic markers for specific colonies are not lost over time, and with highly polymorphic loci, such as microsatellites, there is almost limitless variation among colonies. I will discuss two important applications of genetic fingerprinting of individual colonies: 1.) determination of colony densities and foraging areas around structures; and 2.) evaluation of the colony-level effects of insecticide treatments in the field.

There are few quantitative determinations of the number of subterranean termite colonies occurring around structures for any specific geographic region. Yet such information is critical in determining the relative pressure experienced by different areas and the risk of subterranean termite infestation. To determine the numbers of colonies occurring around structures, and to determine the locations and foraging areas of colonies, we conducted a study on 19 residential properties in the Raleigh, North Carolina area (Parman and Vargo, 2008). We chose houses with current subterranean termite infestations. We installed an average of 69.5 in-ground monitors around each property, with one ring of monitors located close to the foundation wall and concentric rings situated further out in the yard. We also sampled mud tubes in the structure and wood debris out in the yard. Samples were collected monthly for a period of almost 9 months, and all samples collected were genotyped at 10 microsatellite loci after the methods of Vargo (2000) and Dronnet et al. (2004). Across the 19 properties, we identified 188

colonies belonging to three species: *R. flavipes* (89.9% of all colonies), *R. hageni* (7.4%) and *R. virginicus* (Banks) (2.7%). We found an average of 9.4 colonies per property, for a mean ± SD density of 61.8 ± 50.7 colonies per ha (Figure 1). We also determined that the vast majority of colonies were simple families, i.e., headed by a single pair of monogamous reproductives, most likely the founding king and queen (87% in *R. flavipes*, 83% in *R. hageni* and 40% in *R. virginicus*). In addition, for the most part colonies had limited foraging ranges, with a mean linear foraging distance of only 4.4 m in *R. flavipes*. Thus in the Raleigh, NC area, it is quite clear that subterranean termite colonies tend to be fairly localized but can occur in high densities on residential properties, although they can occur at much higher densities in natural areas in the same region, where densities of 125 and 300 colonies per ha have been reported from undisturbed forest sites (DeHeer and Vargo, 2004).

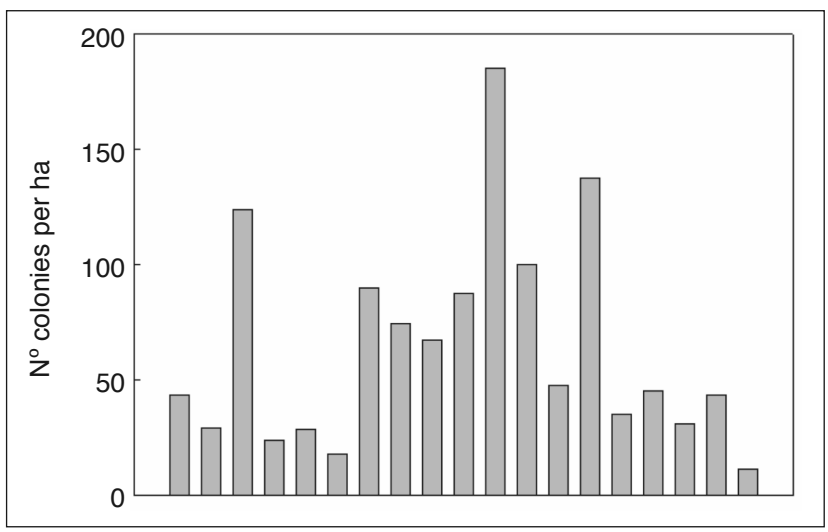

Figure 1. Population densities of subterranean termites in 19 residential buildings with an average property size of 1,854 (Raliegh, NC USA). Abundance: *Reticulitermes flavipes*, 89.9%, *R. hageni*, 7.4%, and *R. virginicus*, 2.7%.

We have also used genetic markers to track the fate of colonies after insecticide treatment in the field. In one study, we determined the ability of hexaflumuron bait (Sentricon®) to eliminate colonies around an apartment complex in Raleigh, NC (Vargo, 2003). In this study involving 234 monitoring stations, we were able to track the fate of 36 subterranean termite colonies for at least 1 yr after baiting. We found that all of the colonies (35 *R. flavipes* and 1 *R. virginicus*) disappeared during the course of the study demonstrating that they were suppressed and likely eliminated by baiting treatment. This study also showed that termite pressure close to the buildings decreased by more than 50% during the 3-yr sampling period. Although there have been many studies involving field trials of hexaflumuron, the number of colonies tracked here is far greater than any other single study previously reported. In a survey of 41 studies conducted up until 2003 using primarily mark-release-recapture methods, the mean number of colonies tracked over time per study was 3.9 ± 4.9 (Vargo, 2003).

More recently, we used genetic fingerprinting of colonies to track the fate of 62 subterranean termite colonies – *R. flavipes*, *R. virginicus*, *R. hageni* and *C. formosanus* - baited with noviflumuron (Recruit IV®, Dow AgroSciences) located around 24 buildings in Florida, Mississippi, Tennessee, Louisiana and Georgia (Thoms et al., 2009). In all cases, treated colonies disappeared from the study areas and were not detected again. More than half the properties (54%) had new termite activity after the first bait cycle, but DNA analysis showed that in all cases these were new colonies that had not been previously baited. The genetic data provided in this study was critical for determining that reappearance of termites in the bait stations was not due to bait failure, but rather due to the migration of new colonies into the areas left vacant by successful colony elimination. These results were instrumental in getting the bait approved for use on new buildings in Florida as part of the newly revised state rules for termiticide efficacy (Thoms et al., 2009). No doubt, the use of genetic markers in efficacy trials will play an increasingly important role in the registration of baits and other termiticides.

We have recently completed two studies examining the colony level effect of nonrepellent liquid termiticides on subterranean termites. Some degree of transfer of nonrepellent active ingredients has been demonstrated in the laboratory and there have been anecdotal reports of colony-wide effects; however, there had not been rigorous tests performed in the field to track colonies after treatment. In both of the studies we conducted, we selected

houses in the Raleigh, NC area with subterranean termite infestations. We installed a grid of in-ground monitoring stations around the houses, and collected samples from the stations, as well as from mud tubes and wood debris in the yards on a monthly basis for 6-9 months. All samples collected were genotyped for colony identification. After this initial pre-treatment period, colonies were treated with either imidacloprid (Premise®) or fipronil (Termidor®) by a licensed pest management professional. We then collected samples monthly for 3 mo and then quarterly for 2 yr in the case of the imidacloprid treatment and 3 yr in the case of the fipronil treatment.

For the imidacloprid treatment, we treated 11 houses (Parman and Vargo, 2010). All but one house was infested by a single colony, while a single house was attacked by two colonies simultaneously. Nine of the 12 colonies disappeared from the study within 90 days of the treatment and were not detected again. These were clearly suppressed and most likely eliminated. In contrast >70% of the colonies out in the yard that were not near the treatment zone continued to be redetected, usually many times. Figure 2 shows a representative house and the locations of colonies before and after treatment. These results are consistent with significant colony level effects of imidacloprid treatment, resulting in strong suppression and likely colony elimination in most cases.

We obtained similar but even more pronounced effects with fipronil. In this study, we used eight houses and monitored the colonies for 3 yr. Three of the houses were infested by two colonies each while the remaining houses were infested by a single colony. In this case, all 11 colonies disappeared by the 90 day post-treatment date and none of them was detected again. Figure 3 shows the colonies around a representative house used in this study. These results show strong colony-level effects of fipronil treatment, resulting in the suppression or elimination of all infesting colonies.

CONCLUSIONS AND FUTURE DEVELOPMENTS

We can expect genetic methods to play an increasingly important role in termite pest management in the future. There will undoubtedly be many contributions in the areas of taxonomy and tracking the spread of invasive species. The use of genetic fingerprinting methods to track colonies will become common place as genotyping techniques become easier and cheaper. This will greatly advance our understanding of the colony-level effects of termiticide treatments in the field, as well as giving us a more accurate picture of the foraging sizes of colonies of various species and their population densities in different geographic regions. In addition to providing powerful tools for understanding termite species and populations, genetic methods are shedding new light on termite development and reproduction, revealing potential new targets for control.

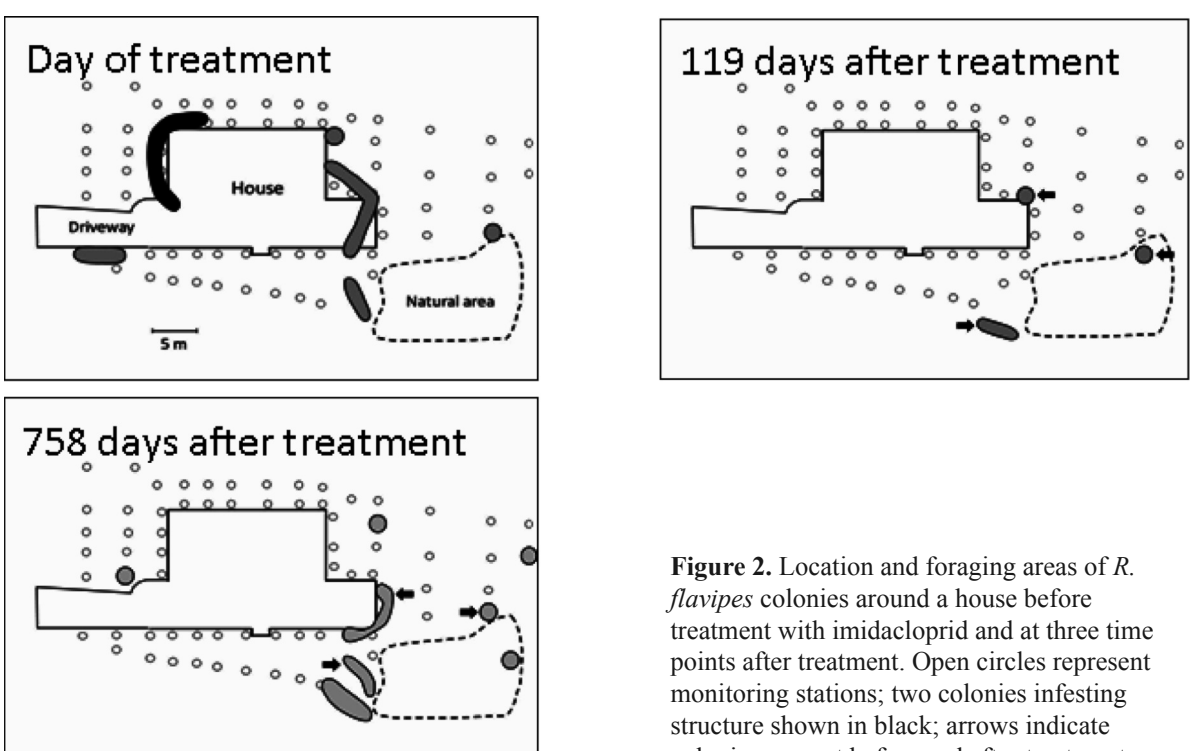

Figure 2. Location and foraging areas of *R. flavipes* colonies around a house before treatment with imidacloprid and at three time points after treatment. Open circles represent monitoring stations; two colonies infesting structure shown in black; arrows indicate colonies present before and after treatment

New targets could include physiological processes in the termites themselves using RNAi technology (Price and Gatehouse, 2008), or the symbiotic gut fauna that are critical in lignocellulose digestion. For example, Zhou et al. (2006) have shown that a hexamerin gene plays a critical role in soldier determination and silencing of this gene by application of RNAi inhibits soldier differentiation. Targeting this gene or other genes involved in development or reproduction could offer a highly specific and environmentally sound way to control termites in the future. The rich gut symbiotic bacterial flora in termites could possibly be exploited as potential shuttle systems for delivering genetic or other control methods to entire colonies. Husseneder and Grace (2005) have transformed naturally occurring bacteria from the gut of *C. formosanus* and successfully re-introduced them into termites. These transformed symbionts quickly spread through the colony via trophallaxis and replication. Thus, such a system offers the opportunity to introduce self-replicating shuttle system to deliver a gene or toxin throughout the colony. Finally, the genome of *Zootermopsis nevadensis* (Hagen) has recently been sequenced, the first termite genome to be decoded (Liebig, Brent, Korb, Vargo, unpubl. data), and there are other termite genomes in the works. The availability of the genetic code of termites will certainly provide an unprecedented view into the fine workings underlying the development, reproduction, behavior and physiology of these important pests and possibility reveal many targets for control.

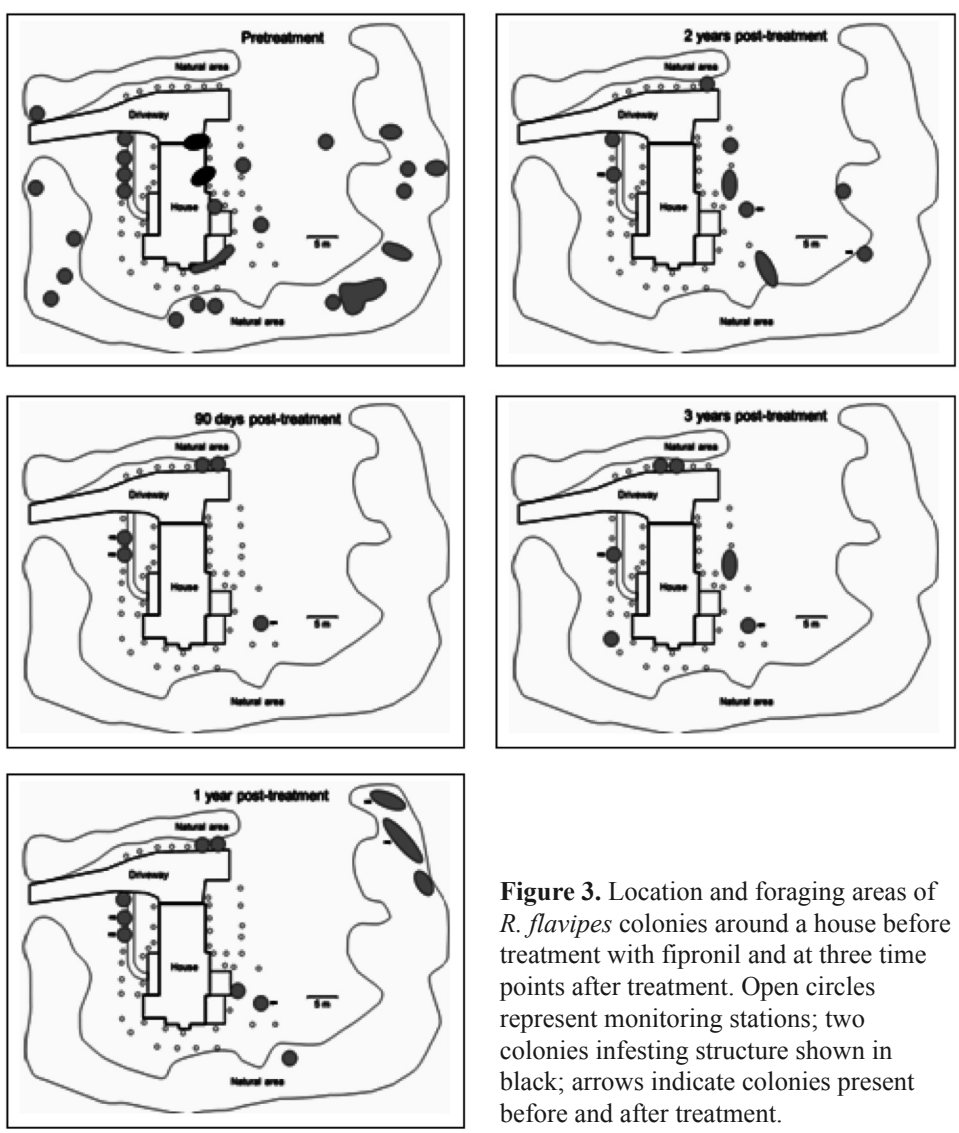

Figure 3. Location and foraging areas of *R. flavipes* colonies around a house before treatment with fipronil and at three time points after treatment. Open circles represent monitoring stations; two colonies infesting structure shown in black; arrows indicate colonies present before and after treatment.

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