

**Mechanisms of Termite Spread in Wisconsin and Potential Consequences as a Result of Changing Climate Trends**

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**ABSTRACT**

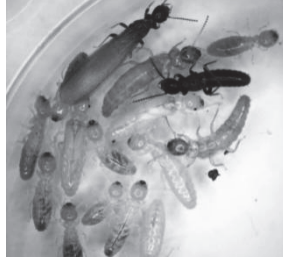
Mature colonies of *Reticulitermes* spp. reproduce and spread mainly by secondary (rather than alate) reproductives throughout their geographical distribution, but especially near the northern boundaries of their range. Historically in Wisconsin, winged reproductives of the one established species, *Reticulitermes flavipes* (Kollar), have been noted only a few times in ground-dwelling colonies and are thus not thought to spread in this manner. Previous research suggests that populations in Wisconsin were introduced by anthropogenic movement of infested materials, a hypothesis supported by recent results from amplified fragment length polymorphism (AFLP) data. Both spatial and non-spatial genetic clustering models suggested strong genetic differentiation among all colonies sampled, indicating little transfer of genetic material. On May 02, 2012 numerous alate reproductives were seen in Janesville, Wisconsin, independent of heated structures after a particularly warm winter. Triggers of alate formation and the influence of temperature in alate differentiation are topics of ongoing research to better assess the risks for future termite introductions, which could rapidly expand the range of *R. flavipes* northward.

**INTRODUCTION**

Subterranean termites cause extensive damage to wood and wood products, with estimated annual damages in the billions of dollars. In North America the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), has a broad distribution and is responsible for much of this cost. This species is most common in southeastern North America but is found as far north as Wisconsin and some parts of Canada.

Success of termite colonies results, in part, from their high level of developmental plasticity. Unlike social Hymenoptera, termites can undergo major phenotypic changes at various stages of development in response to changing colony needs or environmental conditions. In general, *R. flavipes* colonies reproduce by secondary or supplementary reproductives rather than primary, alate reproductives (Howard and Haverty 1980), resulting in large, subterranean populations. This is particularly true of colonies along the northern boundary of their distribution. In Wisconsin, alates have been reported numerous times from heated structures during the spring, but swarming of winged reproductives in the field has never been observed. Esenther (1969) noted alates in ground-dwelling colonies in May and June of 1966-1967, an observation that was not repeated until May 02, 2012, when hundreds of alates were seen at a field site in Janesville, Wisconsin. This observation followed a particularly warm winter, which may have played a role in differentiation into this rarely seen, terminal caste form (Figure 1).

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**Figure 1: Caste diversity in *R. flavipes***

In this study, we document known areas of termite activity in Wisconsin and assess genetic difference among as well as within the various termite colonies using amplified fragment length polymorphism (AFLP). These data can assist in identifying patterns of termite spread in the state. Based on the field observation of alates after a particularly mild winter we hypothesize that differentiation into the winged reproductive caste may be, at least in part, linked to temperature. Changes in global climate trends may alter methods of termite dispersal in Wisconsin, increasing the risk of termite-related damage. This study serves to provide baseline data for understanding dispersal of *R. flavipes* in the northern range of their boundary by examination of population genetic structure.

## METHODS

Termite samples were obtained from 12 locations around Wisconsin as well as from one Mississippi colony to serve as a “genetic out-group.” Total genomic DNA was extracted from the head and thorax of eight workers (near the 3<sup>rd</sup> or 4<sup>th</sup> instar) per collection site followed by digestion using restriction enzymes EcoRI (5’-G|AATTC-3’) and AseI (5’-AT|TAAT-3’). Double-stranded adaptor sequences EcoRI-AD and Bfa-AD, with overhangs complementary to the digested ends, were then ligated to the restriction fragments. Pre-selective PCR amplification of restriction fragments with ligated adaptors was performed with primers EcoRI+G and AseI+G followed by a selective PCR amplification step using primers EcoRI+GG and AseI+GT.

Analysis using DAX 8.0 (Van Mierlo Software, The Netherlands) revealed 155 informative AFLP markers. AFLP-SURV (Vekemans 2002) was then used to calculate polymorphism, expected heterozygosity and  $F_{ST}$  values and GENELAND (Guillot et al. 2005) was used to identify genetic clusters in both spatial and non-spatial models.

## RESULTS

Information from field studies as well as conversations with pest control operators and homeowners provide a clear picture of areas of termite activity in Wisconsin. Observations suggested activity to be limited to the southern half of the state, a trend that was also noted by Esenther (1969). Reported areas of known termite activity are shown in Figure 2.



**Figure 2: Known areas of *R. flavipes* activity in Wisconsin. (\* indicates colonies unconfirmed by authors)**

Calculated  $F_{ST}$  values were used to determine the magnitude of genetic structure (differentiation) among termite colonies sampled in southern Wisconsin. Values close to zero indicate high genetic similarity and values close to one indicate low genetic similarity. Overall  $F_{ST}$  values for the majority of the colonies were larger than expected, suggesting strong genetic differences, which may imply separate origins of introduction.

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Assignment of individuals into genetic clusters with GENELAND supported trends observed in the calculated  $F_{ST}$  values. In nearly all cases, individuals from a collection locality were assigned to the same cluster suggesting a high level of genetic similarity within colonies and high amounts of genetic divergence among colonies. Both spatial and non-spatial GENELAND models provided similar results. The spatial model (i.e. including geographic location) identified 11 separate genetic clusters in 67% of all replicates. Applying UPGMA to estimate indirectly the variation of individual associations in specific clusters indicated that the two LaCrosse colonies were assigned to the same cluster in about 70% of the MCMC replicates. The two Oshkosh colonies were always present in the same cluster, an occurrence consistent with the reduced magnitude of genetic differentiation between them compared to LaCrosse ( $F_{ST}$ = 0.143 and 0.237 respectively). The non-spatial model yields a modal 12 genetic clusters in 29% of all replicates. UPGMA analysis of the non-spatial model indicated distinctive clustering of both the Oshkosh and LaCrosse colonies. Interestingly, exclusion of the spatial component from the model provided some support for genetic relatedness between colonies sampled in Muscoda and Janesville populations, an occurrence not observed in the spatial model.

### DISCUSSION

In this study we identified nine new termite populations in Wisconsin, compared to those previously reported by Esenther (1969). Many known colonies are associated with urban settings but at least two, Janesville and Hazel Green, currently survive independently of heated structures in the southern part of the state. These sites may be the most likely for production of alate swarms in the future.

The large  $F_{ST}$  values and consistent patterns of genetic clustering observed among Wisconsin colonies suggest strong genetic isolation among these termite colonies. This supports the long-standing hypothesis that termites do not disperse through winged alate reproductives in the northern-most ranges and were also likely the result of multiple introductions from more southerly and widely scattered source populations. Data from the two LaCrosse sites and the two Oshkosh sites suggest that some transfer of genetic material occurs between colonies but only within a short geographic range. However, this may also be the result of human movement of infested wood rather than dispersal by alates. Sampling of colonies throughout the southern United States may reconcile this hypothesis. If formation of the alate caste is linked to temperature, dispersal by winged reproductives may increase with shifts in global temperatures, altering the genetic structure and range of *R. flavipes* in Wisconsin.

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