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Section 1 **Biology**

Is there a role for termite alates in colony expansion in Wisconsin?

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ABSTRACT

Termite colonies in Wisconsin tend to be large and widely spread out geographically, and separated by distances up to 1342km. We recently completed a study to determine the genetic diversity and population substructure of thirteen existing colonies of *Reticulitermes flavipes* using amplified fragment length polymorphism to determine patterns of termite dispersal in Wisconsin. Measures of inbreeding, heterozygosity, genetic variation, polymorphism and geographic distances showed that colonies had mixed characteristics of what was expected for colony expansion through budding versus multiple introductions at physically separate sites by means of alates or human movement of infested materials. Overall, these results did not provide evidence of colonies formed by alate breeding pairs. Instead, we hypothesize that *R. flavipes* is likely spread by anthropogenic means, including discarded rail ties, in Wisconsin. Nearly all known areas of termite activity in the state lie on or near major rail lines. A new generation of dual-treatment of crossties with water soluble borates overcoated with a second treatment of water insoluble (oil borne) copper napthanate or creosote may begin to limit transfer of insect colonies via Interstate commerce.

Keywords: *Reticulitermes flavipes*, budding, population structure, alates, genetic variation, climate change, inbreeding depression

1. INTRODUCTION

In most colonies of *Reticulitermes flavipes* (Kollar) within the southeastern United States, alate dispersal and development of brachyptereous neotenics provide one primary means of genetic mixing (Thorne et al 1999). In short, a female alate from one colony finds and mates with a male alate from another, unrelated colony to form a new nest, which usually occurs in the springtime. In Wisconsin, the late spring and early autumn are thought to limit the necessary time frame for outdoor alate formation. Flights that occur indoors are thought to be the result of moderated winter temperature by means of heated structures. These flights tend to occur from early spring into June. Often, this is the most common way that homeowners become aware of a termite infestation. During 50 years of work on *R. flavipes* in Wisconsin, Esenther (1969) never saw a typical alate flight, nor did he ever find a field colony headed by primary reproductive derived from an alate flight. Esenther concluded that in Wisconsin, local spreading of colonies occurs largely through new colony formation by fission (budding) of mature colonies (Thorne et al. 1999). Esenther (1961) also concluded that the introduction of *R. flavipes* across Wisconsin may be associated with rail lines. Further evidence for large budding colonies comes from the observation that treating a

diffuse colony structure over a multi-block area by trap, treat and release at one or two sites can eliminate the infestation over an entire city at one time (Green et al. 2013, Osbrink, et al. 2011).

One of our initial hypotheses was that the lack of alate dispersal in Wisconsin could lead to an inbreeding depression in termite colonies. Inbreeding in *R. flavipes* can be generated in a variety of ways: colonies formed by budding, colonies formed by two sibling alates supplemented by asynchronous swarming and colonies formed by multiple supplementary reproductive neotenics (Reilly 1987). Better insights into initial colony growth will improve our understanding of colony establishment and the spread of invasive species (Janowiecki et al. 2013).

The objectives of our descriptive study was to compile anecdotal evidence for distribution patterns and growth dynamics of *R. flavipes* colonies, and to evaluate if alate flights in Wisconsin have the capacity to initiate new colonies and successfully disperse *R. flavipes*. The principle results of this investigation are that although colonies formed by successful flights of swarming alates (inbred or outbred) are possible it is unlikely that this occurs with any frequency in Wisconsin. We present one field observation that suggests global climate change, particularly shorter winters, could alter this pattern in the future (Peterson 2010).

2. EXPERIMENTAL METHODS

2.1 Discovery of alates in an outdoor colony in Janesville, WI

Groups (25) of newly emerged alates from a single untreated, early-season, field-collected colony were collected from a dead Hickory tree (*Carya. sp*.) in Janesville, WI in May 2012. They were placed into plastic dishes with filter paper and wet paper towels and kept in the dark from May thru September 2012 to show potential for egg laying and production of juveniles. See Table 1. During the interim a maximum of 53 juveniles was present but after that egg production and juvenile populations seemed to drop off and die quickly for unknown reasons or contamination. These pairings indicate the capacity of alate pairs to breed successfully if they can find a suitable environment after flight and dealation. It is not known if inbreeding depression played any role in demise of these small colonies after 6 months or so (Vargo and Hussender 2009).

Table 1: Alate parents (25) seeded from May 12 potential alate population in the field and production of juveniles;
[A representative 90 day period is shown below; -number of dealate parents indicated by "bk"] and eggs.

parents	24-Jul	eggs/	31-Jul	eggs/	23-Aug	4-Sep	7-Sep	10-Sep	24-Sep
1-2bk	0	2	0	0	1	1	1	1	1
2-2bk	9	?	6	0	5	5	6	5	3
3-2bk	8	0	6	0	6	8	8	8	8
4-1b1w	4	0	0	0	0	0	0	0	0
5-2bk	5	0	6	0	5	7	7	0	7
6-4bk	7	0	12	0	12	9	9	7	2
7-3b2w	0	0	0	0	4	0	9	9	0
8-4bk	12	1	17	2	12	21	14	21	14
totals:	45	3	47	2	45	37	53	51	35

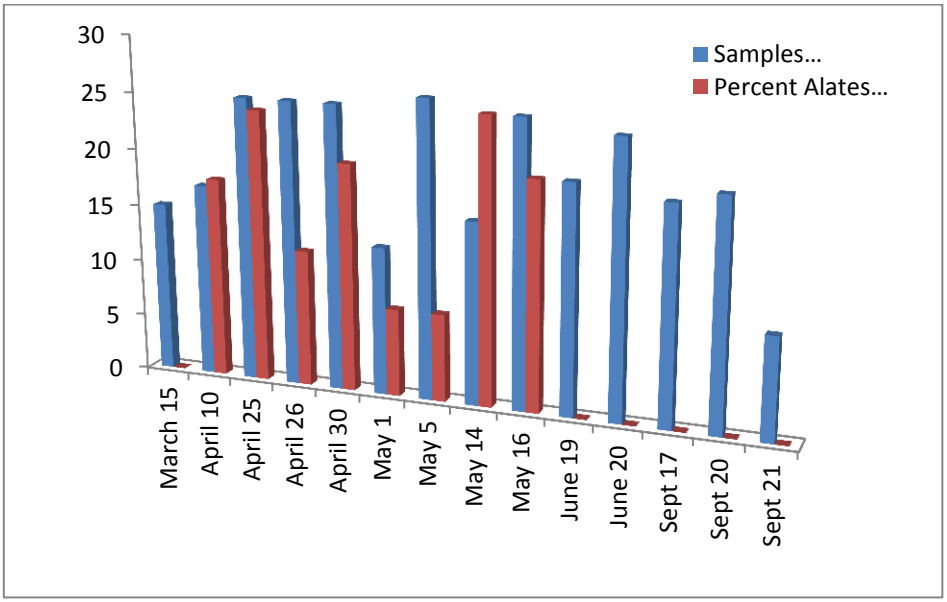
Alate flights in Wisconsin are routinely seen only inside heated homes and wood structures in May and June in the spring. For example, an alate flight was seen inside a house in Madison,

Wisconsin in the spring of 2013. Esenther (1969) reported never seeing an alate flight in Wisconsin out of doors, despite 50 years of work on *R. flavipes* (personal communication). However, rapidly changing temperatures due to global climate change may alter this pattern.

2.2 Observations of alates in Noxubee Wildlife Refuge, Mississippi

In Mississippi, alate flights are more frequent, occurring in the spring during April and May annually. From March to September of 2013, 279 small collections were made of *Reticulitermes* spp. found on the Noxubee Wildlife Refuge (Noxubee, Oktibbeha and Winston Counties, MS). Termites were collected from coarse woody debris of varying size (from logs to small branches) on the refuge. No attempts were made to collect from other locations where termites are sometimes found, such as red imported fire ant (*Solenopsis invicta* Buren) mounds, as described by others (Shelton et al. 1999, 2003). Vials of termites were examined for the presence of fully formed alates and the results presented in the figure below (Fig. 1).

Figure 1: Alate collections from Noxubee Wildlife Refuge, MS



These data suggest that the presence of fully formed alates in colonies of *Reticulitermes* spp. in this part of Mississippi ranges from early April until mid-May, with two peaks in percentage of vials with alates; in late April and mid-May. However, these results should be viewed cautiously, as there was only a single collection period (of only 15 vials)prior to April in 2013. Peak swarming periods have also been monitored in Hawaii for *Coptotermes formosanus* and shown to correlate with low wind speeds(Tong et al. 2013).

3. RESULTS AND DISCUSSION

3.1 *Reticulitermes* colonies in Wisconsin

Esenther (1969) originally described 11 established colonies of *R. flavipes* in Wisconsin. In our survey of 78 pest control companies in Wisconsin in 2010 we received a total of 25 replies of which

only 7 of the respondents had ever seen alates swarming, but always in an urban setting. The majority of alates seen were associated with areas having previously known colonies (Fig. 2). [Two not previously noted were Menasha and Augusta, WI.]

3.2 Neighboring colonies

Colonies that exist in close proximity are known to exist near to the Muscoda, Janesville, La Crosse, and Oshkosh colonies. Colonies in Muscoda and Janesville are known to survive winters without a heated building. In fact, one of our control strategies for the single-cottage Muscoda colony was to turn off the heat all winter long in 2008, but the southern exposure near to the Wisconsin River allowed the colony to survive. The two neighboring houses nearby also became infested but this could easily be due to budding or fission as flights were not required to move such short distances (100 ft =30.5m). Alate flights were only seen indoors at the Muscoda site. The Hazel Green colony of *R. tibialis*, in close proximity to the Illinois border is also able to survive out of doors and could possibly have arrived by alate flights from colonies further south (Arango 2014). No colonies have been found nearby--however its southern location and ability to survive in the wild would be excellent prerequisites to alate swarming.

3.3 Known triggers of alate formation

It has been reported that a combination of climatic factors: humidity, temperature, soil moisture and low winds are required for successful alate swarms (Haverty et al. 2003, Nutting 1969, Vargo and Hussender 2009). Wisconsin is not climatically optimal with long severe winters and late frosts into May, the season for most alate flights further south. However, the spring temperatures in 2012 (Fig. 3) were more than 35°F(19.4°C) above normal for an extended period in March. It is also obvious that daily lows during this two week period were 40°F(22.2°C) above normal lows for that month as well. It is reasonably to speculate that these elevated temperatures triggered the potential for alate flights in May of that year.

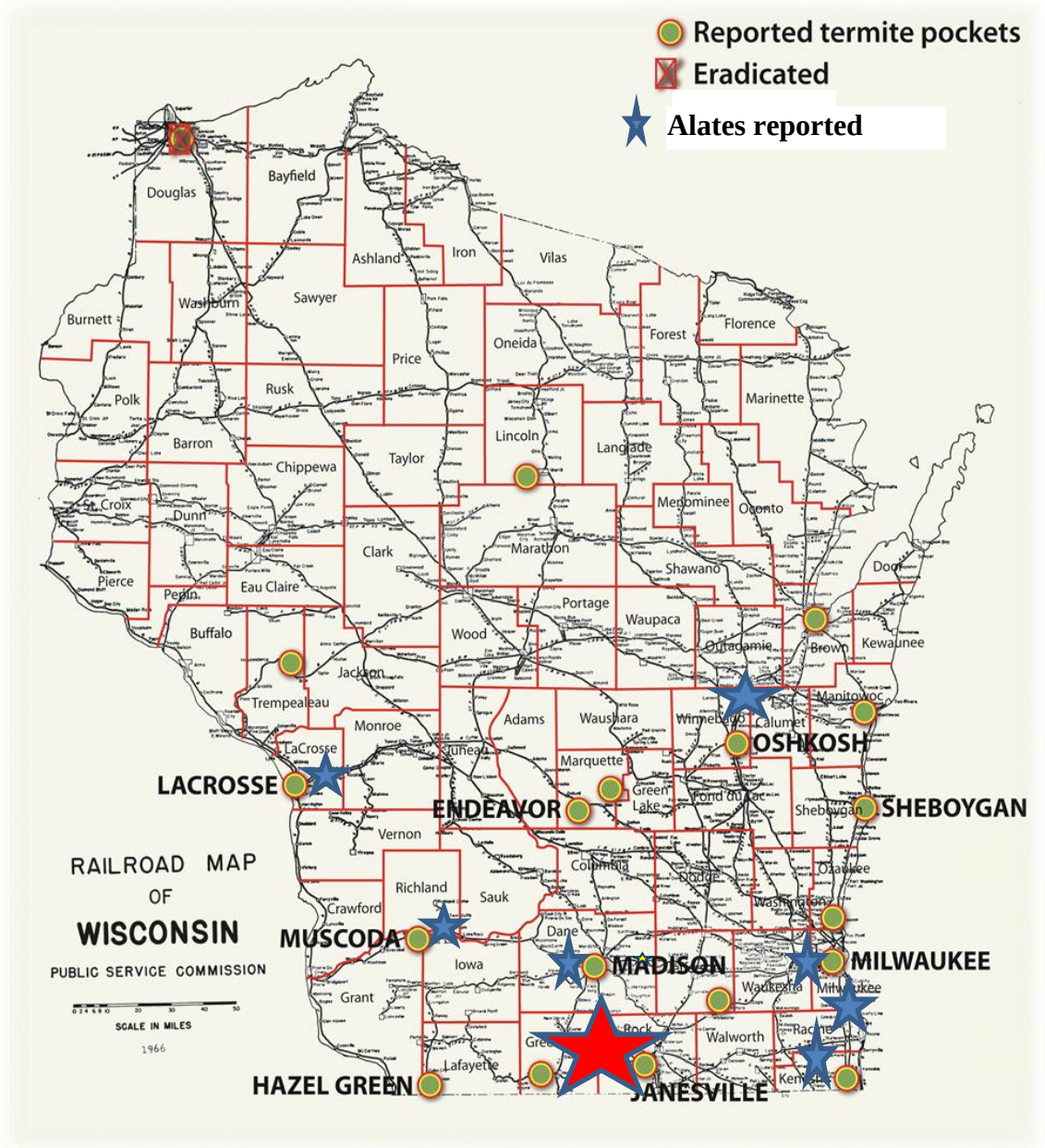


Figure 2: Known termite activities in Wisconsin overlaid on a map of WI rail lines. Hazel Green has been characterized as *Reticulitermes tibialis* (Banks) and all other sites represent *R. flavipes*.

3.4 Distribution and dispersal by railway lines, landscape timbers and infected wood

One of the few major *R. flavipes* sites not on or near a railroad line is the Muscoda site in southwest Wisconsin acrossed the Wisconsin River. However, this site had a retaining wall composed entirely of used railroad ties. Ultimately the owner of this cottage knocked it down and rebuilt it using standards from the State of Hawaii, to include a complete under foundation liquid barrier treatment. The need for an industry response in order to arrest the spread of *C. formosanus* via commercial traffic in used railroad ties was addressed over 10 years ago by Jenkins et al. (2002). More recently, a whole new series of dual treatments of water soluble (diffusible), broad spectrum borates (DOT) have been shown to protect the interiors of crossties from decay and insect damage. This dual treatment reduces the risk of transporting subterranean termites and other insect pests

such as the emerald ash borer (*Agrilus planipennis*, Farimaire) after the crossties come out of service and are shipped for fuel or landscaping timbers (Taylor et al. 2013).

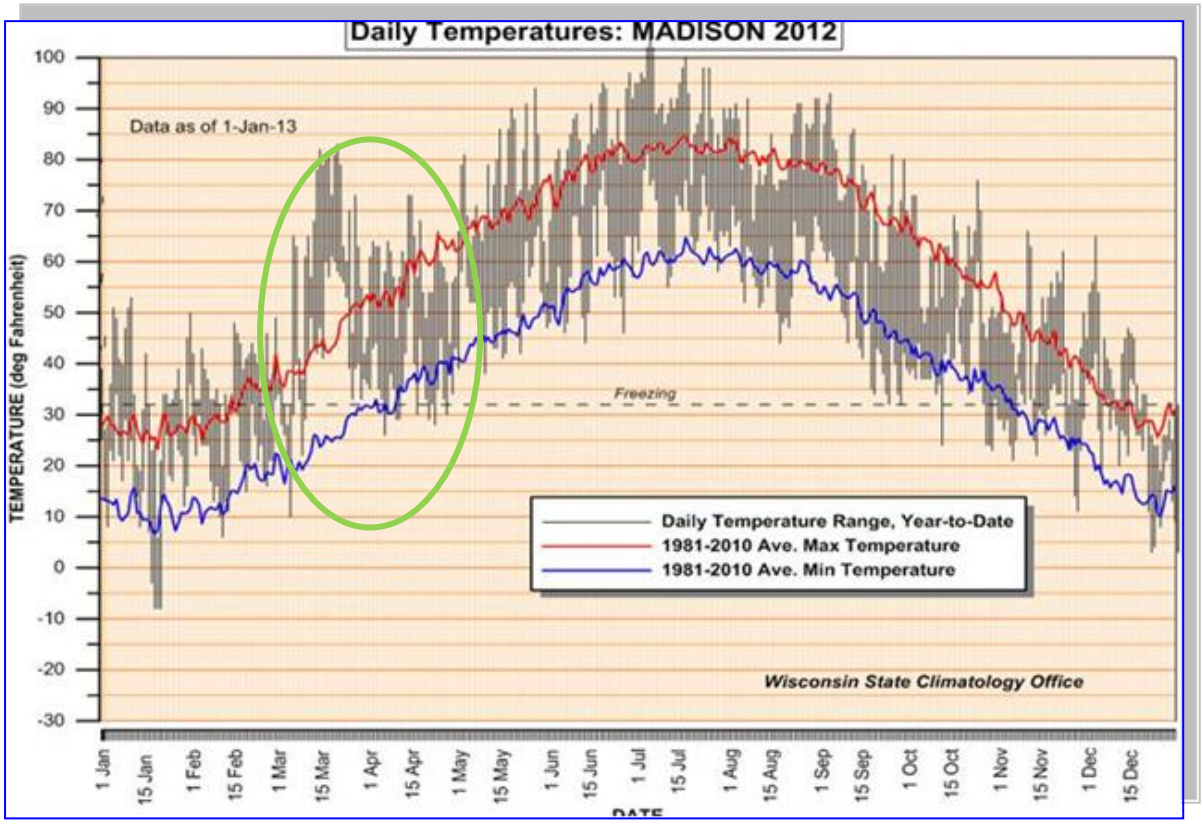


Fig. 3: Daily temperatures for Madison WI 2012 showing abnormally warm period in March 2012 leading up to alate discovery on May 2nd 2012 in Janesville, WI. Note that the low temps for that period are ~35 degrees higher than average lows.

Based upon our genetic study of isolated colonies *of R. flavipes* we hypothesized outcomes for colony structure for dispersal of termites by means of alates vs. budding with secondary reproductives heading the colony (Arango et al. 2014). Of the six criteria used to evaluate colony structure, 3 favored single external introductions or budding and 3 favored multiple introductions or formation by unrelated alate pairs. F_{IS} values represent the coefficient of inbreeding. The two paired colonies in Oshkosh exhibited over twice the level of F_{IS} as other colonies in the study. Heterozygosity represents allelic variation and private alleles* would be found in budding colonies or colonies formed from alate siblings..e.g. brother and sister matings. (*alleles unique to one group of workers). Genetic analyses has shown that inbreeding significantly reduced heterozygosity and allelic diversity (Calleri et al. 2006). F_{ST} values represent differentiation between colonies (Reilly 1987). All F_{ST} pairwise values were significant to 95% confidence levels and are indicative of very high genetic variation. The paired colonies of Oshkosh and LaCross had the lowest F_{ST} values as would be expected with close proximity and as such are not completely isolated at neutral genetic markers (F_{ST}). Wisconsin termite colonies exhibited large within colony polymorphisms (PPL) than expected of geographically isolated colonies thought to be lacking an alate stage.

In light of the very low probability of alate pairings, either siblings or unrelated pairs, the spread of *R. flavipes* in Wisconsin appears to favor i) budding of existing colonies over long distances and ii) anthropogenic transport, with a high probability of transport of infested wood by crossties carried along existing railroads. Low dispersal rates should also promote increased genetic differentiation among colonies (Arango et al. 2014). This confirms that budding should lead to high population viscosity in which colonies near each other are genetically more similar than colonies further apart.

Nevertheless, the observed genetic variability from this study split 50/50 between budding of existing colonies (in spite of isolation by distance) and multiple introductions at the same site. (Arango et al. 2014). The high degree of reproductive plasticity observed in subterranean termites in general, together with the other unique aspects of their ecology, may result in population and colony structures not typically found in other social insects (Bulmer et al. 2001). Subterranean termites (Rhinotermitidae), in particular have exceptionally adaptive breeding systems and dispersal behavior, with a strong tendency to produce neotenics and to form extended family colonies (Vargo and Carlson, 2006). In a study of newly formed tandem-running pairs of both *R. flavipes* and *R. viginicus* alate were found to be significantly related to one another as a result of these pairs forming between nestmates. The proportion of these nestmate pairs was higher in *R. flavipes* (26.1%) vs *R. virginicus* (5.1%). This difference is confirmed by significantly higher estimates of F_{ST} among recently flown *R. flavipes* (DeHeer and Vargo 2006).

4. CONCLUSIONS

Our study found little or no evidence of satellite colonies forming by alate breeding pairs from related or unrelated colonies. However the chances of this outcome were not expected to be very likely. Colony isolation ranging from 0.6 to 283 km did not suggest the likelihood of alate derived colonies of any significance. Alate flights in the genus *Reticultermes* are thought to be limited to less than- 0.458km (Shelton et al. 2006). The two paired colonies in the cities of Oshkosh (0.6km) and LaCrosse (4.5km) were not physically close enough for this pairing even if it conditions were warm enough for alates to swarm. Winged termites are purported to be very weak fliers and have been observed to fly only short distances from their natal nest (DeHeer and Vargo 2006). The potential satellite colonies, even close to the largest known outdoor colony in the state, e.g. Janesville, were more than one mile from the central site and therefore suggest anthropogenic seeding of these sites from mulch distribution at the nearby city compost facility, unless alate pairings were blown by the wind (Myles 2012, 2013, Garcia et al. 2002). The current extreme ranges of climate change currently underway with increasingly warmer temperatures could easily result in initiation of alate swarms and pairings over the next few decades with the potential to dramatically increase the dispersal of *R. flavipes* colonies and termite damage in Wisconsin.

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