# THE EFFECT OF TERMITE BAITING ON THE ECOLOGY AND GENETIC STRUCTURE OF *Coptotermes gestroi* POPULATION

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**by**


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## LIST OF SYMBOLS

| | |
|---|---|
| $ | US Dollar |
| $m^2$ | Square meter (perimeter) |
| % | Percentage |
| cm | Centimeter |
| m | Meter |
| mm | Millimeter |
| ºC | Degree Celsius |
| pH | measure of acidic/basic |
| µL | Microliter |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 16S rRNA | 16S ribosomal RNA |
| AMOVA | Analysis of Molecular Variance |
| ChEBI | Chemical Entities of Biological Interest |
| CI | Confidence Interval |
| COI | Cytochrome Oxidase I |
| COII | Cytochrome Oxidase II |
| CSI | Chitin Synthesis Inhibitor |
| DNA | Deoxyribonucleic acid |
| $F_{IS}$ | the inbreeding coefficient depicted by the correlation within individuals; |
| $F_{IT}$ | the total inbreeding coefficient depicted by the correlation of genes within the total populations |
| $F_{ST}$ | the fixation index implying the degree of differentiation among populations. |
| Hd | Haplotype diversity |
| $H_E$ | Expected heterozygosity |
| $H_O$ | Observed heterozygosity |
| Hp | Haplotype |
| HWE | Hardy-Weinberg equilibrium |
| IBD | Identical by descent |
| LD | Linkage disequilibrium |
| MEGA | Molecular Evolutionary Genetics Analysis |
| ML | Maximum likelihood |
| n | Number of samples |
| $N_A$ | Number of alleles |
| NCBI | National Center for Biotechnology Information |
| NJ | Neighboring-joining |
| P | P-value |
| PCoA | Principal Component Analysis |
| PCR | Polymerase Chain Reaction |
| $\pi$ | Nucleotide diversity |
| PIC | Polymorphic Information Content |
| r | Relatedness coefficient |

| | |
|---|---|
| SD | Standard deviation |
| UMS | Underground Monitoring Station |
| USM | Universiti Sains Malaysia |

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**KESAN UMPAN ANAI-ANAI TERHADAP EKOLOGI DAN STRUKTUR GENETIK POPULASI *Coptotermes gestroi***

## ABSTRAK

*Coptotermes gestroi* dikenali sebagai anai-anai perosak yang menyebabkan gangguan yang ketara melalui aspek ekologi dan ekonomi yang terhadap infrastruktur dan pertanian di seluruh dunia. Tahap keberkesanan bagi kaedah pengumpanan menggunakan Chlorfluazuron, sejenis Perencat Sintesis Kitin (PKI), sebagai bahan aktif telah dilaksanakan dan didapati pembasmian itu berjaya mengawal serangan anai-anai *Coptotermes gestroi*. Walau bagaimanapun, kajian mengenal pasti kesan rawatan pengumpanan anai-anai terhadap struktur genetik dan biologi molekul adalah terhad. Maka, kajian ini dilaksanakan untuk mengkaji kesan rawatan pengumpanan terhadap *C. gestroi* dalam aspek ekologi dan struktur genetik populasi. Matlamat penyelidikan ini meliputi penganalisaan menggunakan urutan mitokondria sitokrom c oksidase subunit I (COI) dan DNA ribosom 16S rRNA untuk menentukan taburan populasi dan hubungan filogenetik enam populasi *C. gestroi* (CG, F, M, S, PK dan B) yang terletak di habitat semula jadi Universiti Sains Malaysia, Pinang. Seterusnya, penanda mikrosatelit khusus spesies telah digunakan untuk menganalisis corak pembiakan dan struktur genetic populasi *C. gestroi* dengan kolorasi faktor persekitaraan dan saiz kawasan pembiakan anai-anai. Perbezaan corak pembiakan dan genetik struktur sebelum dan selepas rawatan umpan anai-anai juga telah dikaji. Hasil kajian menunjukan, terdapat dua haplotip dan tiga kelompok genetik yang berbeza antara enam populasi *C. gestroi* di habitat semula jadi, Universiti Sains Malaysia, Pinang. Secara khususnya, populasi *C. gestroi* mengungkapkan perbezaan genetic sederhana (FST = 0.205) dan pembiakbakaan yang ketara ($F_{IS}$ = 0.456, $F_{IT}$ = 0.573). Manakala, faktor persekitaran seperti suhu dan kelembapan tanah menunjukkan wujudnya hubungan positif terhadap saiz perkembangan kawasan pembiakan *C. gestroi*. Koloni asal *C. gestroi*, terhapus sepenuhnya selepas rawatan pengumpanan dalam kadar masa empat hingga lapan minggu dan didapati tiada rekolonisasi setelah itu. Tambahan pula, selepas rawatan umpan anai-anai, chlorfluazuron, perbezaan genetik yang ketara dan pembiakbakaan negatif telah dilaporkan untuk populasi *C. gestroi* ($F_{ST}$ = 0.468, $F_{IS}$ = -0.490). Kesimpulannya, penyelidikan ini berjaya mengkaji perbezaan ketara terhadap corak pembiakan and structur genetik populasi *C. gestroi* selepas rawatan umpan anai-anai.

**THE EFFECT OF TERMITE BAITING ON THE ECOLOGY AND GENETIC STRUCTURE OF *Coptotermes gestroi* POPULATION**

## ABSTRACT

*Coptotermes gestroi* is a prevalent subterranean termite pest known for significant ecological and economic consequences on infrastructure and cultivated crops worldwide. The efficacy of baits with chlorfluazuron, a type of Chitin Synthesis Inhibitor (CSI), as an active ingredient has been tested against the *Coptotermes gestroi* termites and progressive cessation of termite activity has been established. However, limited studies are available on baiting treatment effect on the genetic structure of subterranean termites. Therefore, this study investigated the breeding pattern and genetic structure of *Coptotermes gestroi* pre and post-baiting treatment with chlorfluazuron bait was investigated. Partial mitochondrial DNA sequences (COI and 16S rRNA) were analyzed to assess the genetic variations and phylogenetic relationship of six different populations (CG, F, M, S, PK, and B) located in natural habitats of Universiti Sains Malaysia (USM), Penang. Next, species-specific microsatellite markers were applied to examine the breeding pattern and genetic structure of *C. gestroi* populations with the correlation of ecological factors and termite territory size. Finally, the variation in breeding patterns and genetic structure before and after the application of baiting treatment was investigated. Within the natural habitat *C. gestroi* population, two haplotypes were detected and three distinct genetic clusters were revealed. Moderate genetic differentiation ($F_{ST}$ = 0.205) and significant inbreeding $F_{IS}$ = 0.456) were exhibited among the populations while environmental factors such as soil temperature and moisture showed a strong positive relationship in the expansion of termite territory size and extended family breeding pattern.

Furthermore, the original colony of *C. gestroi*, completely collapsed after CSI baiting treatment within four to eight weeks, and no recolonization was observed at all sites. Additionally, after baiting treatment significant genetic differentiation with negative inbreeding was reported for the *C. gestroi* population ($F_{ST}$ = 0.468, $F_{IS}$ = - 0.490). To conclude termite baiting showed a significant differentiation in the breeding pattern and population genetic structure of subterranean termites.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

There are over 175 species of termites known to have been discovered in Peninsular Malaysia and among them, *Coptotermes* species from the Rhinotermitidae family is acknowledged as the most significant wood pest with records accounting for $100 million in financial losses in Malaysia due to their considerable destruction towards crops and infrastructure (Darlington, 1994; Lee, 2002, 2021). The *Coptototermes* species commonly known for its subterranean termite features is a eusocial insect that has the ability to forage up to 300 $m^2$, concealing its movement underground with an advanced caste structure consisting of reproductives and infertile castes (Ab Majid and Ahmad, 2015; Thorne et al., 1999).

Not surprisingly, Malaysia, an exemplary temperate region in Asia with a tropical setting ideal for subterranean foraging, frequently experiences significant infestation by *Coptotermes* spp. Biologically, *Coptotermes* attack susceptible living trees found in forests and plantations by consuming core parts of the trees. Records show that *Coptotermes* was responsible for the loss of 74% of trees in forestry (Jasmi, 2016). In urban settings, this species is relatively found foraging through the base of man-made constructions or they spread to buildings from existing vegetation nearby. In Peninsular Malaysia, infestation in buildings by *Coptoterme*s cost approximately $100 million excluding the repair and control cost (Lee, 2021).

Even though most of the time the pest issue by mosquitoes is often overlooked over termite infestation, this invasive pest is still the biggest nightmare for most homeowners and plantation owners. The millions worth of property damage per year in Malaysia is a grave problem for the country's economy. The damage caused by termites is usually not covered by insurance and unlike fire and flood, infestation by termites is very common and is not discovered until there is permanent destruction in a given area. Hence, knowledge of *Coptotermes t*ermite infestation through further research studies will provide a better understanding of the regulation of this invasive pest.

Pest management strategies commonly applied against subterranean species in Malaysia are the application of liquid termiticide and baiting systems (Broadbent, 2011; Oi, 2022). The usage of liquid termiticide has been debated due to an uprise in health and environmental hazards by the termiticide (Su, 2002). Therefore, an alternative resort, bait application with Chitin Synthesis Inhibitors (CSI) has been adopted (Evans and Iqbal, 2015; Lee, 2002). In recent years, CSI baits have impacted termite colony elimination efficiently as it is safer to handle and more 'environmentally friendly' (Peters and Fitzgerald, 2003; Su, 2019). Particularly for *Coptotermes* species eradication, baits with chlorfluazuron, a type of CSI, as an active ingredient have shown progressive cessation of termite activity (Manzoor and Pervez, 2016; Rojo and Acda, 2019; Wan Syahir and Ab Majid, 2021; Wan Umar and Ab Majid, 2020).

The study of the invasive biology of termites through molecular genetics is a multidisciplinary approach to studying termite distribution and population structure in Malaysia. The collaborative approach using behavioral and ecology studies coupled with genetic analysis has provided insights into the mechanism of success for the dispersal of subterranean termites (Ab Majid et al., 2017; Husseneder et al., 2003; Lee et al., 2005; Lim et al., 2021).

## 1.2 Problem Statement

Recent reports have shown that the effect of termite baiting has not only caused successful total colony elimination in weeks but there were differences observed in the caste distribution and colony size after baiting. Treatment with various bait strategies had shown a different effect on the colony foraging behavior. There is a lack of studies discussing the factors that may lead to such changes in the colonies. Besides, the effectiveness of the baiting strategy also depends on the attraction of the initial colony towards the bait, the slow-acting transfer effect on the colony by the bait, abiotic and biotic factors, and under no circumstance's reinfestation of the initial colony after elimination at the treated site. Furthermore, subterranean termite infestation still exists with new colonies invading old and new sites after eradication activities. This requires the need to understand in depth the foraging pattern of subterranean termites using the molecular genetic basis of invasive pest traits.

There are several studies on the effectiveness of baiting treatment against *Coptotermes* species. Unfortunately, there is lack of research on the effect of baiting on the genetic structure of subterranean termites before and after the baiting treatment. Most records only gather information on the termite activity and period of colony elimination but no information in molecular stance on the genetic variation occurring due to baiting treatment within and among the treated colonies.

The molecular information aspect that is readily available on the *Coptotermes* species is mostly on the phylogenetics of the species found around Malaysia and worldwide. The lack of research focusing on populations in a spatial scale area and habitat-specific in Malaysia is concerning as there is no molecular data to infer the degree of relatedness of the termite reproductives and colonies found in the same area or focusing on a certain region. There is previous research on population genetic studies on *Coptotermes* species that provided basic genetic distance information of termite populations from wide geographic regions such as Penang Island, Kuala Lumpur, Singapore, and Taiwan (Yeap et al., 2011). However, there is no publication on the genetic diversity and population structure of *Coptotermes* species concentrating on one single locality in Malaysia.

## 1.3 Significance of study

The research contributes to knowledge of the application of molecular analysis to biology and ecological studies of subterranean termites. An interdisciplinary approach to research is becoming more common between molecular ecology and applied fields to completely understand an organism, genotype, phylogenetics, and environmental variables in which it is crucial to be exploited simultaneously. Molecular genetic data allows entomologists to quantify genetic diversity and differentiation of populations and identify evolutions within both natural and urban invasive pest populations. In the field of urban entomology, pest control methods can be improved through more extensive data obtained through genetic molecular analysis.

Genetic markers have fast-tracked the process of identifying species origin, potential evolution in invasive populations, and the fate of colonies after pest control treatment. Principally, genetic analysis plays an important role in the registration of termiticide efficacy as it provides evidence of the total colony elimination of the original colony and reappearance of termite colonies at treated grounds after eradication is hardly due to failure in bait treatment but because of migration of new colonies into the vacant site. Also, genetic studies on subterranean termites provide information on the foraging pattern and distribution of the social insects, hence providing foresight on the forthcoming plausible area of infestation. Studies on baiting treatment effect on population genetics of subterranean termites that are habitat-specific and focusing on spatial scale regions are important as they will aid in the enhancement of pest management strategies.

### 1.4 Hypothesis

The species identification, phylogeny, genetic variances including haplotype and nucleotide diversity, and pairwise genetic differences between the *C. gestroi* population found in the natural habitats were determined using mitochondrial markers. Differentiation in the breeding pattern and population genetic structure within and among the colonies were assessed using microsatellite markers. The effect of environmental factors on the termite territory size and breeding pattern of the termite population was analyzed. The effect of termite baiting on the *C. gestroi* population pre- and post-treatment was determined. In the current study, the original colony of the *C. gestroi* population completely collapsed due to the consumption of chlorfluazuron baits within four to eight weeks. The polymorphism in the breeding pattern and genetic structure of termite colonies pre-treatment and post-treatment was compared to understand the effect of baiting treatment on the *C. gestroi* population structure.

### 1.5 Objective

This study aimed to address the effect of termite baiting on the ecology and population genetic structure of the *Coptotermes gestroi* population found in Universiti Sains Malaysia, Penang. In order to achieve the aim, the study was constructed with a number specific objectives, which are listed below:

Objective 1 (Chapter 3): To determine *Coptotermes gestroi* genetic variations and phylogenetic relationship by using two classes of mitochondrial markers, cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA).

Objective 2 (Chapter 4): To examine the breeding patterns and population genetic structure of the *Coptotermes gestroi* population using microsatellite markers with the correlation of ecological factors and termite territory size.

Objective 3 (Chapter 5): To investigate differences in the breeding pattern and genetic structure of the *Coptotermes gestroi* population before and after the application of termite baiting.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Subterranean termites

Commonly known as ground-inhabiting (hypogeal) and soil-borne social insects, subterranean termites are the most prevalent termite pests in multiple regions worldwide (Rust and Su, 2012). The subterranean termites establish tunnels beneath the ground, form extensive nesting and underground galleries with a relatively high number of termite colony members, and often forage long distances. The large colony and extensive foraging distances imply high feeding damage as they forage around obstructions and reach the food sources of wood material (Highley, 2004; Sornnuwat et al., 1996; Su, 2001; Su and Lee, 2008). This is a concern as the introduced subterranean species unlike the native species, are not well adapted to the changes in new environments thus likely causing significant damage to man-made infrastructure (Krishna, 2023). Out of 3, 000 known termite species as serious pests, subterranean termites account for 66 out of 79 species uncovered (Rust and Su, 2012).

Primarily, in urban settings, the subterranean termites gain access to buildings through the foundations, and the prominent signs reported are visible mud tubes built over surfaces and the swarming of alates (Ab Majid and Ahmad, 2009). The termites were also found to respond to disturbance through vibrational stimuli such as knocking (Hu et al., 2003). In certain circumstances the infestations and termite galleries are concealed beneath wood surfaces, making observation difficult and only noticed when the structure collapses from critical damage.

Apart from urban settings, subterranean termites are generally accepted insect species in natural woodland habitats. In nature, the subterranean termites associate positive impacts as natural decomposers of fallen trees, stumps, and other decaying wood materials. The termites aid in cellulosic digestion, ensure soil porosity through tunneling, enrich soil with nutrients for vegetative growth, and influence the biodiversity of microbial and animals (Evans et al., 2013; Jouquet et al., 2011; Lavelle et al., 2006; Myer and Forschler, 2019). However, in some cases, few subterranean termite species do contribute to forest tree deterioration. Significant damage was reported on several native and exotic trees with no signs of injuries superficially but internal hollowing of tree trunks and hampering water absorption by nesting in plant root systems by subterranean termite species (Chiu et al., 2016; Jasmi, 2016; Lee, 2014; Zorzenon and Campos, 2015).

The mound-building subterranean species otherwise categorized as higher termite species, *Macrotermes gilvus*, *Macrotermes carbonarius*, *Globitermes sulphureus*, *Microtermes pakistanicus*, and *Microcerotermes* spp. and *Odontotermes* spp. are usually garden termites that are less destructive and secondary pest that often attack plants under stress (Kirton and Azmi, 2005). Conversely, lower subterranean termite species, *Coptotermes* species, are significant wood pest species in urban and suburban settings. The *Coptotermes* species is a tropical species that does not build a mound. They belong to the family Rhinotermitidae and are among the most species-rich genera of all lower termites (Kambhampati and Eggleton, 2000). The genus is estimated to have originated in Southeast Asia. The wide distribution of the species outside native ranges occurred by infested cargo, ships, boats, and also human-mediated land transportation of infested materials (Evans et al., 2012; Gay, 1967). The *Coptotermes* destruction has been reported in the Southern United States (Scheffrahn and Su, 2005), Australia (Lo et al., 2006), Hawaii (Grace et al., 2002), Brazil (Constantino, 2002), China (Wang et al., 2002), Taiwan (Li, 2009), Southern Japan (Vargo et al., 2003), Philippines and Marina Island (Acda, 2004), Malaysia (Darlington, 1994; Lee, 2002), Thailand (Sornnuwat et al., 1996) and India (Roonwal et al., 1989). The relative incidence of *Coptotermes* spp. shown to have increased over subtropical and temperate regions worldwide due to the increase in urbanization and globalized human activity (Evans et al., 2013; Li et al., 2010). In Peninsular Malaysia, six *Coptotermes* species were discovered and among them, *Coptotermes gestroi* (Wasmann) was recognized as the most destructive species (Darlington, 1994; Kirton and Azmi, 2005).

## 2.2 Asian subterranean termites*: Coptotermes gestroi* (Wassmann)

### 2.2.1 *Coptotermes gestroi* history

*Coptotermes gestroi* was first described by the researcher Wasmann (1896) and later was given the common name of Asian subterranean termites by Kirton and Brown (2003). *Coptotermes gestroi* was often misidentified by several junior synonyms such *as Coptotermes havilandi* (Holmgren), *Coptotermes vastator* (Philippine milk termite), and *Coptotermes helmi* (Wassman) due to persistent discovery of the same species in taxonomic literature and genetic synonymity (Kirton and Brown, 2003). The species was also misidentified with other valid *Coptotermes* species such as *Coptotermes travians* (Haviland) in Southeast Asia and *Coptotermes formosanus* in Shiraki, Guam (Su and Scheffrahn, 1998; Yeap et al., 2007, 2010). Nevertheless, *Coptotermes gestroi* was recognized as a primary subterranean pest species originating from Southeast Asia.

The distribution of *C. gestroi* was reported across Southeast Asia (Northeast of India, Myanmar, Thailand, Laos Cambodia, Vietnam, Malaysia, Singapore, and Indonesia) extending beyond its native range into the Philippines, Taiwan, Fiji, and Hawaii, into the New World tropics including Brazil, Florida, Mexico, the Caribbean, Micronesia, the great Antilles and Polynesia (Evans et al., 2013). The first occurrence in Europe was recorded in Italy after a yacht visiting from the Caribbean Islands was found to be infested with *C. gestroi* (Ghesini et al., 2011). Anthropogenic dispersal through human transportation was the primary means of dispersal by *C. gestroi*. Biological characteristics of feeding on sound wood, nesting in the wood, and producing neotenics ease the dispersal activity of *C. gestroi*. Large wooden material used for shipping, soil from planting containers (flower trade), and timber are some materials found to be infested onboard marine vessels and alates were found swarming from infested ships reaching lands (Hochmair and Scheffrahn, 2010).

In Malaysia, *C. gestroi* is an economically important pest. The termite problem is a major issue accounted for by residential premises in Malaysia as new housing projects are developed every year in newly deforested sites and in suburban regions surrounded by forestry. It is also concerning as the current building law enactment in Malaysia has not made it compulsory for the developers to do pre-construction inspections or pest control operations (Lee, 2002). There was a news article on *Focus Malaysia* written by Kenni Ang Joo Koon, a member of the National House Buyers Association (HBA) and lawyer, and Datuk Chang Kim Loong, the honorary secretary general of the non-government organization, mentioned the availability collateral warranty given to housing developers who engage specialist contractors for termite treatment but it typically is an extended warranty and usually the property purchasers are not disclosed on the existence of the warranty (Kenni and Chang, 2021). Besides, in Peninsular Malaysia severe loss in agriculture crops and urban forestry especially timber industry, rubber plantation, and conifer plantations were recorded due to *Coptotermes* infestation. It was usually directed to *Coptotermes curvignathus* but it was reported that *C. gestroi* was also involved (Sajap, 2009).

### 2.2.2 The taxonomy of *Coptotermes gestroi*

The scientific taxonomic classification of *Coptotermes gestroi* (Wasmann) according to the Integrated Taxonomy Information System (ITIS) is as follows:

| | |
|---|---|
| Kingdom | : Animalia |
| Phylum | : Arthropoda |
| Class | : Insecta |
| Order | : Blattodea |
| Superfamily | : Blattoidea |
| Family | : Rhinotermitidae |
| Genus | : *Coptotermes* |
| Species | : *Coptotermes gestroi* (Wasmann, 1896) |

### 2.2.3 *Coptotermes gestroi* morphology

*Coptotermes* species are best identified by the well-developed dome-like frons with large openings known as the fontanelle with one bristle on each side. The head capsule is shaped like a teardrop. The pair of setae around the fontanelle discriminates *C. gestroi* species from other *Coptotermes* species (Wikantyoso et al., 2021). Superficially, the soldier's head is reddish yellow, the legs and abdomen are pale white, and the mandibles are reddish brown and are moderately curved apically. The prominent feature is the secretion of white latex from the fontanelle when the soldier is disturbed. Features of *C. gestroi* workers are more subtle with a yellow-white head capsule and pale-yellow abdominal and legs. The workers are slightly smaller than soldiers. The alates of *C. gestroi* have a head, pronotum, and abdomen of dark brown color and two antennal spots that are vividly visible. The length of the wings is about 13 to 14 mm (Ahmad, 1965; Scheffrahn and Su, 2000). The soldier of *C. gestroi* is shown in Plate 2.1.


Plate 2.1 The soldier of *C. gestroi*

## 2.3 *Coptotermes gestroi* foraging mechanism

Subterranean termites build a ramified tunnel system beneath the ground to forage for food (wood). According to Arab et al., (2012), the foraging begins with workers initiating the exploration for food sources, followed by a trail of pheromones on the pathway, and ends with the recruitment of nestmates from the nest to the feeding sites. The foraging behavior of subterranean termites is highly cryptic and constrained by the environmental factors that the foragers depend on for dispersal. The soil, temperature, rainfall, moisture, and infestation sources influence the foraging strategy of subterranean termites. The variation in environmental factors may also contribute to the changes in termite foraging behavior (Manzoor et al., 2013; Santos et al., 2010). According to previous findings, the maximum depth of foraging activity by *C. gestroi* was recorded to reach a depth of 90 cm (Chutibhapakorn et al. 2000; Manzoor et al., 2013). The foraging distance by *C. gestroi* was recorded as approximately 30 m and the foraging territory was up to 300 $m^2$ in urban settings of Penang, Malaysia (Ab Majid and Ahmad, 2015).

Moreover, a study by Hapukotuwa and Grace, (2014), described *C. gestroi* tunneling behavior as a highly branched network with adequate distance due to the homogenous distribution of wood sources in tropical settings. Tunnels are built in a way to direct the termite colony straight to the area of the food resources. Previous studies have also explored the foraging efficiency of *C. gestroi* in heterogeneous landscapes. The studies revealed foraging efficiency was maximized by the numerous tunnel branching and more intersections at food resources to sustain energy lost through foraging further from the nest (Jeon et al., 2010; Lee and Su, 2009; Lima and Costa-Leonardo, 2012). In addition, the success in foraging by *C. gestroi* was also reflected in the caste composition. *C. gestroi* are eusocial organisms known for their well-organized colonies of soldier-worker interaction and a breeding pattern consisting of multiple reproductives. The survival of the foragers was also directed at their adaptive behaviors such as reproducing in massive numbers, cannibalism during food scarcity, and agonistic behavior toward other insect species or predators (Haifig et al., 2018; Li, Yang, et al., 2010).

### 2.3.1 Effect of environmental factors on foraging mechanism

Notably, environmental resources drive the colony's foraging activity, propagation, and survival. The optimization of environmental factors influences the colony size, the colony demography, age, and its status of reproductivity. The colony sustains growth through seasonal variations, potentially producing more neotenics during droughts and floods to avoid colony collapse (Chouvenc et al., 2022). *C. gestroi* regulate the nest condition and flight activities primarily according to the environmental variables: temperature, moisture, and soil pH. Additional variables, such as soil type and source of infestations; the tree type are also factors that contribute to the nesting and foraging behavior of *C. gestroi*.

Principally, temperature influence on *C. gestroi* is associated with juvenile hormone levels on the regulation of worker-to-soldier proportion. According to previous research higher soldier proportions were recorded when the temperature was favorable (Liu et al., 2009). This shows temperature plays a great part in caste differentiation. Besides, most infestations by *C. gestroi* can be traced in temperate zones suggesting the species is highly tolerant to heat. A high number of *C. gestroi* termites and wood consumption was recorded when the temperature range was around 33.6-38.6 6ºC (Cao and Su, 2016). In contrary. In low temperatures (< 20ºC) the termites were immobilized or their movement slowed. Temperature also plays a key factor that leads to the risk of man-made infrastructure invasion by *C. gestroi* species because most indoor spaces in buildings are favorable with indoor heating or room temperature increasing the opportunity for the termite colony to naturalize and expand in non-native settings (Li et al., 2013).

Moisture is a fundamental factor for the *Coptotermes* termitarium structural development. *C. gestroi* built the shelter tubes and branched tunnels using moisture from the soil. Hence extensive tunnelling by *C. gestroi* colony is recorded when the soil moisture content is high. Additionally, high moisture level aids in wood consumption and against dehydration. It was also reported that moisture helps the termites stay hidden from predators by reducing their walking sound (Oberst et al., 2019; Wong and Lee, 2010). Due to *C. gestroi* termite's ability to maintain moisture within the nest relative humidity has a limited effect on the colony survival as moisture retained in the nest indirectly influences the relative humidity. Nevertheless, relative humidity does affect the survival of alates that live in the nest. Seasonal humidity is significant for alate dispersal as the species is highly susceptible to desiccation. Hence, alate distribution is common after rainfall as they survive better in high relative humidity with readily available water resources (Gautam and Henderson, 2011; Kulis et al., 2008; Wiltz, 2012; Zukowski and Su, 2017).

Besides, the soil environment has a significant impact on the lower termites. The aspects of soil profile such as pH and soil texture are important as they determine the termite activity in infested sites. Researchers have reported that *Coptotermes* species prefer an acidic environment (pH < 7) and the pH of the nest was usually higher than the surrounding soils. However, the soil pH was not a limiting factor for *C. gestroi* attacks. As their infestation increases the content of nitrogen and phosphorus in the infested grounds improves the soil for termite activity (Arif et al., 2019; Arinana et al., 2020). Based on soil analysis, *Coptotermes* termites build mud tubes by depositing layers of sand and clay. Sand and clay have water retention properties and *Coptotermes* termites use these soil elements mixed with their saliva and feces for the construction of in-ground galleries (Cornelius and Osbrink, 2010). Moreover, clay is preferred by termites as wood underload is supported by layers of clay walls as the termite colony consumes wood from beneath (Oberst et al., 2019). Aside from that, in urban sites, *Coptotermes* infestation was reported at infrastructures built upon loamy sand or sandy loam, a combination of sand, silt, and clay for water retention and support for the colony to forage further into the structure while in tropical sites *Coptotermes* infestation was found in soil with rich in organic matter such as potting mix, peat moss and mulch (Cornelius and Osbrink, 2010; Majid and Ahmad, 2013).

An additional factor that needs to be considered is the sources of infestation in natural habitats especially the type of tree susceptible for *C. gestroi* infestation. The information is crucial to eliminate sources of infestation from urban forestry and loss of trees in forest canopy. In Malaysia, oil palm, rubber (*Hevea brasiliensis*), conifer, and Acacia plantations were highlighted to be affected by *Coptotermes* species in the early years of establishment (Kalshoven, 1963). Later, the attack was observed on larger tree species, Araucaria trees in botanical gardens (Jasmi and Hassan, 2011). Followed by commonly thriving agriculture plantations such as mango and coconut trees (Kalshoven, 1963). Recent research studies reported *C. gestroi* infestation on Pine trees in the urban canopy (Chouvenc and Foley IV, 2018; Zorzenon and Campos, 2015). These tree species were observed to be infested several times in patchy or isolated form and the damage was severe. Once the trees are removed by deforestation, large wood remnants are left on the forest floor as a food resource for the termites which aid the colony to expand further in the search of food leading to the infestation of infrastructures (Chouvenc and Foley IV, 2018; Kirton et al., 1999).

### 2.3.2 *Coptotermes gestroi* breeding pattern

*Coptotermes gestroi* termites adapt a eusocial life pattern which has three key elements: (1) reproductions are skewed to reproductive division and non-reproductives are completely sterile, (2) cooperative care over offspring and (3) overlap of adult generation in the nest (Anderson, 1984; Thorne, 2003). Fundamentally, the *C. gestroi* develop with monogamous pair primary reproductives (monogamy). The primary reproductives in the form of alates initially use internal nutritional resources to establish the first cohort of offspring, mostly workers. The first cohort helps with the colony's growth and acts as alloparents (Chouvenc, 2022). The production of neotenic (secondary reproductives) serves as either a replacement for primary reproductives or supplementary reproductives in order to rapidly grow and optimize expansive foraging ranges (Costa-Leonardo and Arab, 2004). The other castes, workers, and soldiers help to maintain the colony's food resources and protect the nest. Research has also mentioned caste determination was influenced by juvenile hormones which induce the molting of workers into soldiers. It is also proposed that juvenile hormones are influenced by extrinsic factors such as the environment (Vargo and Husseneder, 2009).

The breeding pattern of *Coptotermes* species has remained elusive due to cryptic nesting and foraging habits. Nevertheless, due to increased attention from entomologists on the biology and dispersal pattern of this group and with the aid of molecular genetic markers informational insights have been acquired on the colony breeding structure. During *Coptotermes* colony foundation, the colony was composed of a simple family breeding pattern. A simple family consists of a pair of primary reproductives and their offspring. An extended family colony is produced when one or both primary reproductives are replaced by secondary reproductives within the colony. The increase in related reproductives causes the increase of inbreeding within the colony. In addition, to simple- and extended-family colonies, mixed-family colonies occur when different colonies fuse to form one colony or during the expansion of the colony, there were multiple unrelated reproductives present (Vargo and Husseneder, 2009). Furthermore, even though the *Coptotermes* colonies lack agonistic behavior, most of the time the colonies in introduced ranges from either distinct simple family colonies or extended family colonies derived from simple families (Aguero et al., 2020; Vargo, 2019; Zhang and Evans, 2017).

## 2.4 Economic importance

Social insects especially, termites are one of the leading pests worldwide. *C. gestroi* is recognized as an economically important structural pest and also one of the invasive species among 28 termite species that have expanded beyond its native range (Su and Scheffrahn, 2000). Consequently, the global burden keeps increasing as climate change is contributing to the rapid dispersal and hybridization of invasive species.

Research findings have revealed that a colony of 2000,000 termites can feed approximately 5.4kg of wood per year and an average colony of *C. gestroi* consists of 1.13 to 2.75 million individuals (Govorushko, 2019; Sornnuwat et al., 1996; Su, 2002). Which led to a global economic impact of $40 billion annually by subterranean termites (Rust and Su, 2012). In southeast Asia, the damage estimated by *C. gestroi* was $400 million per year (Lee et al., 2007). Recently, the damage cost recorded solely in Malaysia was $100 million and is estimated to be higher if repair and control are included (Lee, 2021). In Malaysia, subterranean termites account for 50% of business turnover for the Malaysian pest control industry which is around $8-10 million. Proportionally, 65% of termite control measures were provided for residential premises, 20% for industrial zones, 10% for commercial buildings, and 5% for other locations (Lee, 2002). It is estimated the global industry market for insect pest control was valued at $15.23 billion in 2020 and is expected to increase by 5.2% by 2030. Additionally, Doggett and Lee, (2020) suggested that during the pandemic, urban pests thrived due to an increase in poverty and restrictions to pest control management, thus post-pandemic it is expected for the pest management industry to face the global economic fortune of exterminating pests, especially termite infestations.

## 2.5 Overview on termite control management

Basically, for decades the termite control methods consisted of physical barriers and chemical control using liquid termiticide as the standard against termites (Kirton and Wong, 2001). In Malaysia as well liquid termiticide was predominantly accepted as a termite prevention and control measure, nevertheless, termite baiting gained attention after the liquid termiticides were reported as eco-unfriendly and were not a permanent solution against subterranean termites. Besides, another alternative termite control measure was also introduced, non-chemical control more commonly known as biological control. Biological control unfortunately was less successful toward subterranean termite eradication due to their cryptic foraging behavior and eusocial breeding pattern. Hence baiting treatment was adopted as a stand-alone, sustainable, and more environmentally responsible strategy (Broadbent, 2011; Oi, 2022).

## 2.6 Chemical control

### 2.6.1 Liquid termiticide (repellent and non-repellent termiticide)

The organophosphates and pyrethroids were the pioneer liquid termiticides available for subterranean termites' prevention and control. The organophosphates served as less persistent chemical control and were widely accepted before the 2000s. However, its toxicity to vertebrates led to its immediate ban from the market. Soon after pyrethroids (bifenthrin, cypermethrin, fenvalerate, and permethrin) took over as the primary termiticide with features of prolonged residual effect, effective at low concentrations and low mammalian toxicity. Pyrethroids are repellent versions of liquid termiticide that deter termite foragers away from treated sites. This was concerning as subterranean termites had the foraging strategy to effectively detect, and avoid the treated sites and were able to access the sites through the untreated gaps. In addition, environmental issues on pyrethroids' toxic effect on non-target organisms caused less of its usage and the search for better alternatives (Kuswanto et al., 2015; Rust, 2014; Su, 2011).

Hence, non-repellent liquid termiticides (fipronil, imidacloprid, chlorfenapyr, and chlorantraniliprole) were introduced as an improvement over the pyrethroids. The non-repellent termiticide acts as a slow-acting toxicant and serves as a termiticide barrier. Termite colony suppression has been effective using non-repellent termiticides and studies have reported a reduction in the foraging range of termites before total colony elimination. The application of the termiticide involves drilling holes into the foundation around the perimeter of the structure. This allows the termites to be exposed to termiticide when they try to invade through the foundation. However, there are limitations to the non-repellent termiticide. For instance, application is limited depending on the structure's foundation. Clay soil element beneath the foundation interferes with the distribution of termiticides. Moreover, the liquid termiticide does not last long in the soil and degrades over time which leads to the reapplication of the product. This is troublesome as the drilling for the reapplication of termiticide cause damage to the infrastructure. Additionally, the non-repellent termiticide acts as a barrier hence there is no effect on the colonies outside the perimeter of the treated site which can causes the subterranean termites to infest other structures near the treated sites and reinfest treated site after there is no residual of the termiticide (Chouvenc, 2018; Kuswanto et al., 2015; Neoh et al., 2014; Rust, 2014; Su, 2011).

### 2.6.2 Termite baiting

Termite baiting is a successfully applied subterranean termite control strategy worldwide. The primary goal of termite baiting is colony elimination with minimal application of active ingredients (Oi, 2022). The fundamental approach for termite baiting is to attract termites into in-ground or above-ground stations using blank wood pieces. Once termite infestations are recognized, wood pieces are replaced with bait toxicants. Bait stations are usually made of plastic and have bait substances consisting of cellulose material with active ingredients. Above-ground bait stations are installed directly on termite mud tubes or the infested region so the termites can access them easily and consume the bait immediately. In-ground stations are mostly cylindrical stations with a disk top that are planted in the soil. The disk top allows easier identification during the inspection and prevents the station from sinking into the ground. The stations are also well secured and can only be accessed using a key provided by the manufacturer to unlock the disk top cover. The in-ground stations are usually made of plastic and have perforated sides to allow the termites to enter the station to access or wood or bait in the station (Cabrera et al., 1969).

Termite baiting is applied as a preventive measure in new constructions for structural protection (Oi, 2022). The key to termite baiting success was its baits property as slow-acting, non-repellent towards subterranean termites, and dose-independent lethal time. Furthermore, the baiting strategy is to target colony elimination through the transfer of toxicants among the colony members instead of the mortality of individual termites (Evans and Iqbal, 2015; Grace and Su, 2001; Su and Scheffrahn, 1993).

Therefore, insect growth regulators were added as active ingredients in the bait matrices. Su and Scheffrahn (1993), reported the potential of Chitin Synthesis Inhibitors (CSI), a type of Insect growth regulator that prevents termite metamorphosis. The termite workers when exposed to CSI bait move to the central nest and fail to moult. This led to the mortality of affected termites amid primary reproductives. This causes the reproductives to move, only to be surrounded by death termites. Eventually, the colony collapses after the primary reproductives are eliminated due to starvation (Su, 2019). Baiting using CSI has been the most triumphant strategy against *Coptotermes* species. *Coptotermes* termites experienced defects in caste differentiation as high soldier numbers and low workers were recorded after exposure to bait. The observed worker-soldier ratio suggested functional death of the colony (Chouvenc and Su, 2017; Messenger et al., 2005).

The pioneer CSI bait that was introduced was hexaflumuron which was the first successful commercialized termite bait for subterranean termite eradication.

Hexaflumuron baits were introduced using the Sentricon system, a type of in-ground baiting system, and 98% success rates were reported after field applications against *Coptotermes* species. Soon after other CSI baits were introduced to the market, including noviflumuron (Sentricon), diflubenzuron (Advance), novaluron (Trelona), bistifluron (Xterm), and chlorfluazuron (Exterra) (Lee, 2002; Su, 2019). To date, three different bait systems that have records for effectively reducing termite infestation in Malaysian field trials: The Sentricon Colony Elimination System with hexaflumuron bait by Dow AgroSciences (Koehler et al., 2021), the Exterra Termite Interception and Baiting System with chlorfluazuron bait by Ensystex (Wan Umar and Ab Majid, 2020) and the Xterm Termite Baiting System with bistifluron by Sumitomo Chemical Co, Ltd. (Lee, 2007). Among them efficacy of chlorfluazuron bait in colony elimination of *C. gestroi*, through area-wide baiting program showed promising results.

### 2.6.3 Chlorfluazuron

According to Chemical Entities of Biological Interest (ChEBI), chlorfluazuron is a member of the benzoylphenyl urea group insecticide and its primary mode of action is to inhibit chitin synthesis within the insect integument. Chlorfluazuron was first incorporated in the bait matrix for testing against subterranean termites by Rojas and Morales-Ramos, (2001) after its effectiveness as a chitin synthesis inhibitor against *Coleoptera* species. The findings reported an evident decline in termite activity after ingesting chlorfluazuron and total mortality of termite colonies within nine weeks. Shortly after, Peters and Fitzgerald, (2003) investigated the termiticidal activity of chlorfluazuron for the management of *Coptotermes acinaformis*. With a single application of bait matrix, colony elimination was reported within twelve weeks and no evidence of repellence was recorded. Several studies have documented the eradication of *Coptotermes* species using chlorfluazuron (Osbrink et al., 2011; Rojo and Acda, 2019; Wan Syahir and Ab Majid, 2021; Wan Umar and Ab Majid, 2020). The summary of the research findings was consistent with minimal application of bait product through selective baiting (less than 300g of bait), successful colony elimination within four to twelve weeks, and efficient area-wide control against *Coptotermes* species.

## 2.7 Physical barrier

Physical barriers consist of sheet materials of solid metal such as stainless steel wire mesh and particle materials such as granite and basalts as foundations of constructions. Pre-construction, installation of the physical barriers are performed beneath the concrete slabs to prevent termites from tunneling and penetrating the structures. The installation of the barriers is easier, environmentally friendly, and remains effective for an indefinite period. The physical barriers are designed to prevent termites from entering the structure but it is possible the termites detected in the vicinity of the structure (Acda, 2018; J. Hu et al., 2006).

## 2.8 Biological control

Several preliminary studies are being carried out on biological controls against termites. The biological control agents are usually in the form of botanicals, predators, parasites, and pathogens (Mishra et al., 2021; Verma et al., 2009). The emphasis on biological agents is due to its potential of non-chemical measures against termites which is safer and environmentally friendly. In Malaysia, a previous study by Jessica et al., (2019), reported entomopathogenic fungus isolates against *Coptotermes* termites caused complete mycosis of the termites within eight days of exposure, showing its potential as a promising biological control agent. Nevertheless, the biological control strategy is still new and needs to be explored more to determine its efficacy against subterranean termites. There are concerns of a negative impact on non-targeted organisms by infection or parasitism. In a review by Chouvenc et al., (2011), on the fifty years of attempted biological control of termites, it was suggested the potential of termite biological control has essentially failed and is not applicable for commercial termite control due to unrealistic optimism in studies, publication bias and poor understanding of termite biology.

## 2.9 *Coptotermes gestroi* molecular genetics

*C. gestroi* has been the main subject of molecular genetic investigation to date. Molecular genetic studies involve molecular markers consisting of DNA sequences or proteins that can be screened for polymorphism to reveal genetic variations. Termite identification based on morphology may not be reliable and could be misleading, hence molecular markers are efficient tools for precise and rapid identification of species as well as to understand the diversity and gene flow within and among populations (Husseneder et al., 2003). Earlier studies on *C. gestroi* focused on tracking foraging activity and determining its distribution as native or introduced species around the world through phylogenetic studies (Jenkins et al., 2002; Li, 2009; Yeap et al., 2007, 2010). The genetic studies involved mitochondrial markers to build species-level phylogenetic trees and phylogeography illuminating the maternal lineage of *C. gestroi*. Hence, there is a series of mitochondrial DNA sequences for *C. gestroi* published in the National Center for Biotechnology Information (NCBI) and mostly associated with 16S ribosomal RNA (16SrRNA), cytochrome oxidase I (COI) and cytochrome oxidase II (COII) gene markers. Most of the phylogenetic studies were focused on connecting *C. gestroi* termites found widespread from ecologically varied geographies of the world and limited studies focused on habitat-specific phylogenetic relationships of *C. gestroi* colonies (Ab Majid et al., 2017; Alajmi et al., 2019; Gentz et al., 2008; Jenkins et al.,