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Assessment of genetic markers for the determination of *Coptotermes formosanus* × *Coptotermes gestroi* (Isoptera: Rhinotermitidae) F1 hybrids

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The Formosan subterranean termite *Coptotermes formosanus* Shiraki and the Asian subterranean termite *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae) are 2 of the most invasive subterranean termite species in the world (Evans et al. 2013; Chouvenc et al. 2016a). These species are allopatric in their native area, but their distributions now overlap in a few locations with a subtropical climate, including Taiwan, Hawaii, Hainan, and south Florida (Grace 2014; Cao & Su 2015). Although both species are genetically distinct and the 2 lineages evolved independently for approximately 18 million yr (Bourguignon et al. 2015), it was recently shown that they had the potential for hybridization in Florida (Chouvenc et al. 2015). Interspecies mating between alates of both species was observed in the field in 2013, 2014, 2015, 2016, and 2017, and incipient F1 colonies were successfully established in the laboratory (T. Chouvenc, University of Florida, Institute of Food and Agricultural Sciences, Ft. Lauderdale Research and Education Center, Ft. Lauderdale, Florida). However, it is unknown if such F1 hybrids are established in the field, primarily because subterranean termites have a cryptic nest and the soldier morphology is highly conserved within the group (Scheffrahn & Su 2005), preventing rapid detection and identification from field samples.

These species have been introduced into Florida (1980–1990s), and we suspected that the potential for hybridization may have been limited to the past few years because the geographical overlap was first recorded in 2005 (Chouvenc et al. 2016b) and the first simultaneous dispersal flight was recorded in 2013. Currently, there are no reliable morphological markers to identify hybrids. Therefore, genetic markers that would allow for testing the potential hybridization in the field are needed. *Coptotermes* colonies mature 8 yr after initial foundation (Chouvenc & Su 2014), which implies that the detection of field F1 hybrid colonies may only be possible years after the initial interspecies mating, and it may take decades before F2 may be recorded, if ever produced.

Gene flow among populations can be detected using microsatellite markers to determine if introgression events occurred in the past (Gaggiotti et al. 1999). The use of nuclear markers provides insight about the mating structures within a population that mitochondrial markers cannot, because the latter only provide information on maternal lineages. Creating a genetic library of nuclear markers for both *C. gestroi* and *C. formosanus* at overlapping locations would provide the background genetic information required to test for the detection of F1 hybrids as a diagnostic tool, with an initial emphasis on south Florida populations, the only location where interspecies mating was confirmed. However, the different genetic makeup of the 2 parental species implies that the nuclear markers used for genetic determination must be compatible for both species and their hybrids. Over the past few years, several studies have developed microsatellite primers to investigate genetic population structures of various *Coptotermes* species (Thompson et al. 2000; Vargo & Henderson 2000; Yeap et al. 2011; Liu et al. 2012) but it is unknown if a marker developed for one species would be compatible with another species and their potential hybrids.

We screened 42 microsatellite primers previously developed for *Coptotermes* and obtained a list of nuclear markers that can be used interchangeably among F1 individuals resulting from all mating combinations. Alates of *C. formosanus* and *C. gestroi* were collected during simultaneous swarming events in 2014 in Ft. Lauderdale, Florida. Pairings of males and females were placed in individual rearing units as described in Chouvenc et al. (2014), and all mating combinations were used for the establishment of incipient colonies: conspecific colonies (♀*C. gestroi* × ♂*C. gestroi*, ♀*C. formosanus* × ♂*C. formosanus*) and heterospecific colonies (♀*C. gestroi* × ♂*C. formosanus*, ♀*C. formosanus* × ♂*C. gestroi*). After 1 yr of rearing and colony growth in the laboratory, 5 workers from 5 colonies of each mating combination were sampled and processed for DNA extraction, as described in Chouvenc et al. (2015). In addition, 12 field samples from each parental species collected throughout south Florida were added to our laboratory samples to confirm that the alleles identified from our laboratory colonies matches the genetic diversity in the field.

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Table 1. List of 6 nuclear markers that successfully displayed different size alleles in both termite species, *Coptotermes formosanus* and *Coptotermes gestroi,* and their hybrids. These markers can be used to build a standard allele library and as a diagnostic tool to detect potential F1 hybrid individuals from the field.

Locus	Reference	Primer sequence[a]	Motif	T_a (°C)[b]	Target Qubit (ng/µL)	Allele size (bp) *C. gestroi*	Allele size (bp) *C. formosanus*
CopF6	Liu et al. 2012	F: CAGTGGCAGCGACGTATA R: ATCCTGGAGTCCTAAGAAGC	$(AC)_8GC(AC)_{14}$	56.9	1.5	168, 174	176, 184
CopF14	Liu et al. 2012	F: CTACAAGGCTACCATCAGG R: GGAACAGCGAGACGAGAT	$(CT)_{13}$	55.0	0.7	194	208, 226
CopF10	Liu et al. 2012	F: AGGTGTTGAATGGGCTGTT R: CCAAGCCTGCCAGAAAGT	$(AC)_{17}$	61.4	1.5	302	326
Cg33	Yeap et al. 2011	F: TTTCATCGAAAGTGCAGGTG R: TGTCGCATGAGGAAGATGTC	$(CAAA)_{16}$	56.0	1.5	202, 205, 208, 211	193
CF10-4	Vargo & Henderson 2000	F: GCGCATGTGGACTGTAAAAA R: TCCAAGTATGCTGATCGGGT	$(AGT)_{22}$	61.4	3.0	162, 165, 168, 171	126, 150, 153
Clac1	Thompson et al. 2000	F: CAGAGGTGACATCAGAAATTG R: GCACATAACAGTAAACCTGCTG	$(AG)_9AA(AG)_4$	53.0	1.5	186, 172, 175	191

Allele sizes displayed represent observed values from12 specimens from each species collected in south Florida.
[a]F = forward primer, R = reverse primer
[b]T_a = annealing temperature

All 42 microsatellite primers were tested and optimized for PCR amplification from 4 original studies (Thompson et al. 2000; Vargo & Henderson 2000; Yeap et al 2011; Liu et al 2012). The primers were subjected to a series of gradient polymerase chain reactions (PCRs) to determine the best annealing temperature that would amplify products from both *Coptotermes* species as well as their respective hybrids. The PCRs were comprised of standard *Taq* buffer (New England Biolabs, Inc., Ipswich, Massachusetts), 1.25 U *Taq* DNA polymerase (New England Biolabs, Inc., Ipswich, Massachusetts), 200 mM each dNTP, 0.4 µM each primer, 2 µL of template DNA, and sterile molecular grade water to a final reaction volume of 50 µL. The microsatellite loci were amplified with either Mastercycler Gradient Thermocycler (Eppendorf North America, Hauppauge, New York) or Arktik Thermocycler (Thermo Fisher Scientific, Inc., Waltham, Massachusetts) using the following cycling conditions: initial denaturation step at 95 °C (90 s), followed by 34 cycles at 95 °C (30 s), annealing at 53 °C to 61.4 °C (60 s), 72 °C (2 min), and a final extension at 72 °C (8 min). Amplification products (5 µL) were separated on an 8% polyacrylamide gel using electrophoresis, stained with ethidium bromide and visualized using UV illumination.

Upon analysis, 6 primer pairs successfully provided polymorphic alleles for genotyping individuals from the 2 *Coptotermes* species and their F1 hybrids where the allele size was different in each parental species but expressed jointly in F1 hybrids. The forward primers of all 6 primer pairs were fluorescently tagged with 6-carboxyfluorescein, and the PCR was repeated. The resulting products were purified using the Wizard® PCR Preps DNA Purification System (Promega Corporation, Madison, Wisconsin) and run on an 8% acrylamide gel to check purity and prepare samples for genotyping. The amount of product amplified was quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, California). The dilution factor for each primer pair was optimized by serially diluting (10^1–10^4) the amplified products and selecting the dilution factor that gave the best result for genotyping. The annealing temperatures, target Qubit values, and the genotyping results for the 6 markers are summarized in Table 1.

This study provides a diagnostic tool for rapid detection of F1 hybrid termites from field populations in south Florida. In the years to come, any samples collected in areas with overlapping distribution in south Florida will be tested. We will expand this approach to Taiwanese populations in the near future using the same markers to determine the range of alleles from these populations and check for the presence of F1 in the field. Both species have been established in Taiwan for a much longer time than in Florida (Li et al. 2010), which implies that if hybridization also occurs there, it may be easier to detect (Su et al 2017).

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Summary

This study investigated nuclear markers in *Coptotermes formosanus* Shiraki and *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae) that can be used as a diagnostic tool to detect F1 hybrids from field samples. Six microsatellite markers were compatible for both parental species and hybrid termites and were optimized so that a standard gene library can be built for the south Florida *Coptotermes* populations.

Key Words: termite; microsatellite; interspecies; optimization

Sumario

Este estudio investigó marcadores nucleares en *Coptotermes gestroi* y *C. formosanus* que pueden ser utilizados como una herramienta de diagnóstico para detectar híbridos de F_1 a partir de muestras de campo. Seis marcadores de microsatélites fueron compatibles tanto para las especies parentales y las termitas híbridas y se optimizaron para que una biblioteca de genes estándar pueda ser construida para poblaciones de *Coptotermes*en el sur de la Florida.

Palabras Clave: termita; microsatélite; intraespecies; mejoramiento

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