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Short communication

Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of *Gp-9* alleles

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Summary. Oligonucleotide primers were designed to discriminate between the *Gp-9*^{B} and *Gp-9*^{b} alleles found in monogyne and polygyne colonies of fire ant, *Solenopsis invicta* Buren. Primers specific for the *Gp-9*^{B} allele produced a 517 bp amplicon and primers specific for *Gp-9*^{b} allele produced a 423 bp amplicon. When both sets of primers were multiplexed, homozygous monogyne ants produced a single 517 bp amplicon (specific for *Gp-9*^{B}), whereas heterozygous polygyne ants produced one 517 bp amplicon and one 423 bp amplicon (specific for *Gp-9*^{B} and *Gp-9*^{b}, respectively) which allowed the *Gp-9* alleles to be discerned in a single reaction. This method was tested on ants from 20 monogyne colonies and 20 polygyne colonies and was 100% accurate in discriminating the two forms.

Key words: Monogyne, polygyne, *Solenopsis invicta*, *Gp-9.*

Introduction

The red imported fire ant, *Solenopsis invicta* Buren, exists in two socially distinct forms. Colonies of the monogyne form are characterized by having a single fertile queen, while the polygyne form contains multiple fertile queens per colony (Glancey et al., 1973). Ross and Keller (1998) and Krieger and Ross (2002) have described a gene (*Gp-9*) apparently responsible for the existence of the 2 social forms exhibited by *S. invicta*. Monogyne queens were homozygous, possessing only the *Gp-9*^{B} allele, whereas polygyne queens were heterozygous, possessing *Gp-9*^{B} and *Gp-9*^{b} alleles (Ross and Keller, 1998).

We exploited nucleotide substitutions between the *Gp-9* alleles to develop primers specific for each allele (*Gp-9*^{B} and *Gp-9*^{b}). The resulting multiplex polymerase chain reaction (PCR) method is able to discriminate *Gp-9*^{Bb} alleles in a single reaction.

Methods

Genomic DNA was prepared from polygyne and monogyne queens (n = 15 and 15 individual queens) and males (n = 5 and 5 individual males), and groups of 10-20 worker ants (n = 5 and 5) from different colonies of *S. invicta* collected from 29 locations in north central Florida. As individual workers in a polygyne colony exhibit 1 of the 3 genotypes (BB, Bb, or bb), DNA purification was conducted with a group of at least 10 workers to minimize the possibility of falsely scoring a polygyne colony as monogyne. Ant colonies were identified as monogyne by the presence of a large physogastric queen of more than 20 mg wet weight (Porter, 1992). Polygyne colonies were identified by the presence of two or more inseminated queens with degenerated wing muscles (Porter, 1992). Queen samples were collected in 2001 and worker samples were collected in 1992 as part of a study of polygyne stability (Porter, 1993). All queen and worker samples were preserved in 70% isopropanol. Genomic DNA was extracted as described by Valles et al. (2002). Primers were designed by aligning the *Gp-9*^{B} (accession numbers: AF427893, AF427894, AF427897, AF459414) and *Gp-9*^{b} (accession numbers: AF427898, 427899) alleles from GenBank with the Vector NTI 7.1 program (Informax, Inc., Bethesda, MD) and choosing areas unique to each allele. *Gp-9*^{B}-specific primers corresponded to positions 1683-1703 (26BS: 5′CTCGCCGATTCTAACGAAGGA) and 2167-2199 (16BAS: 5′ATGTATACTTTAAAGCATTCCTAATATTTTGTC). *Gp-9*^{b}-specific primers corresponded to positions 1307-1334 (24bS: 5′TGGAGCTGATTATGATGAAGAGAAAATA) and 1702-1729 (25bAS: 5′GCTGTTTTTAATTGCATTTCTTATGCAG).

Multiplex PCR was conducted by the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94 °C for 2 min, then 35 cycles at 94 °C for 15 sec, 55 °C for 15 sec, and 68 °C for 30 sec, followed by a final elongation step of 5 min at 68 °C. The reaction was conducted in a 50 µl volume containing 2 mM $MgCl_2$, 200 µM dNTP mix, 1 unit of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of primers 24bS, 25bAS, 26BS, and 16BAS, and 1 µl of the genomic DNA preparation (50 to 500 ng). PCR products (12 µl) were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, positive and negative controls were run alongside treatments.

The *Gp-9*^{B} amplicon was cloned from polygyne and monogyne queens and the *Gp-9*^{b} amplicon was cloned from polygyne queens. Amplicons were ligated into pGEM-T Easy and transformed into JM109 competent cells (Promega, Madison, WI). Insert-positive clones were identified and DNA sequences of inserts were elucidated by the University of Florida, Interdisciplinary Center for Biotechnology Research (Gainesville, FL).

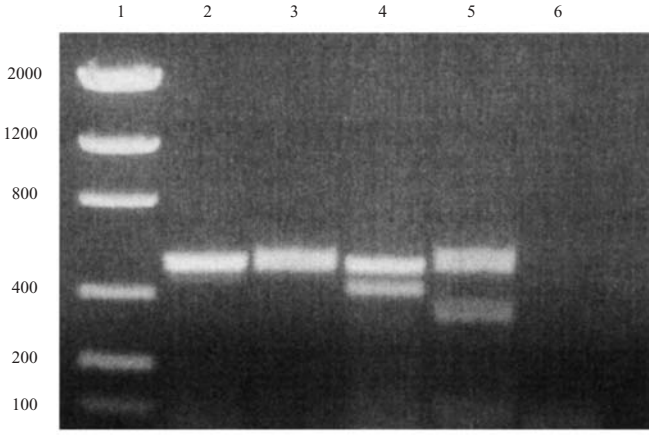

Figure 1. Banding patterns on a 1% agarose gel after multiplex PCR with *Gp-9* allele-specific primers. Column 1, molecular weight markers expressed as base pairs (bp); column 2, PCR conducted with monogyne queen genomic DNA in the presence of primers 24bS, 25bAS, 26BS, and 16BAS; column 3, amplicon from lane 2 (*Gp-9^B* allele-specific amplicon) digested with *Hin*dIII; column 4, PCR conducted with polygynous queen genomic DNA in the presence of primers 24bS, 25bAS, 26BS, and 16BAS; column 5, amplicons from lane 4 (*Gp-9^B and Gp-9^b* allele-specific amplicons) digested with *Hin*dIII; column 6, negative control

Results

The *Gp-9^B*-specific primers produced a 517 bp amplicon from monogyne and polygyne queens and workers (Fig. 1). Primers specific for *Gp-9^b* allele produced a 423 bp amplicon exclusively from polygyne queens and workers. When both sets of primers were multiplexed, monogyne ants produced a single 517 bp amplicon, whereas polygyne ants produced 2 amplicons (517 and 423 bp). This pattern was 100% consistent among all of the samples we evaluated (n = 15 for queens, n = 5 for workers for each social form). Furthermore, the alleles (B and b) were able to be discerned in haploid males and sterile diploid males. As anticipated, restriction digestion with *Hin*dIII reduced the *Gp-9^b* amplicon to 2 fragments (335 and 88 bp) while the *Gp-9^B* amplicon was uncut (Fig. 1). Sequences of each amplicon (*Gp-9^B* and *Gp-9^b*) were found to be identical to each corresponding allele as reported previously (Krieger and Ross, 2002).

Discussion

Two nucleotide mismatches within the last 4 nucleotides of the 3′ terminal end of a primer are known to reduce PCR yield dramatically (Kwok et al., 1990). Primers 24bS and 25bAS were identical matches for the *Gp-9^b* allele, but mismatched the *Gp-9^B* allele by 1 and 2 base pairs at the 3′ terminal end of each primer, respectively. Primers 24bS and 25bAS produced a 423 bp amplicon exclusively from polygynous ants. Correspondingly, primers 26BS and 16BAS were identical matches for the *Gp-9^B* allele found in polygyne and monogyne individuals. Primer 26BS specificity was conferred by 2 nucleotide mismatches in the *Gp-9^b* template on the 3′ terminus. Primer 16BAS was an identical match for both alleles, *Gp-9^B* and *Gp-9^b*. These multiple base pair mismatches at the 3′ termini of the primers conferred the specificity responsible for the discrimination of each allele by multiplex PCR (Fig. 1).

The multiplex PCR method was completed in a single reaction with advantages of being rapid, specific, nondestructive toward the colony, and accomplished with archived samples stored in alcohol. The method provided 100% accurate discrimination of polygyny with a small sample of workers. The procedure can be completed in 3–4 hours and costs about $3 per sample when run in a group of 10.

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