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# CAP-PCR Useful for Analyzing the Kin Relatedness of a Small Ant Species

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Since Hamilton [1] stressed the importance of kin selection for the evolution of altruism in animals, kin relatedness has been one of the major issues in animal ecology and sociobiology. Among various animals, ants, bees, and wasps have been the main study organisms in the argument on social evolution; in particular, sex ratio has been considered to be a primary issue to show the effect of kin selection on the evolution of eusociality in these insects. Since Trivers and Hare [2] predicted the investment ratio of females to males in Hymenoptera to be 3:1 under worker control and 1:1 under queen control, a large number of studies have been made to date [3]. Because their prediction assumes monogyny and monandry (single mating), some authors have developed techniques for estimating the insemination frequency of queens from relatedness among their daughters. Although Craig and Crozier [4], Pamilo [5], and many authors [6] investigated kin relatedness by using allozymes, this method hardly detects enough genetic variation to exactly determine nestmate relatedness [7]. Therefore, Heinze et al. [8] used multilocus DNA fingerprinting for detecting a sufficient genetic variation in the ant *Camponotus floridanus.* The multilocus DNA fingerprinting needs a comparatively large amount of DNA for each individual and its application is restricted to large ant species only [8]. This means that the DNA analysis cannot be replicated for each individual. This handicap was overcome by several studies [9] using the random amplified polymorphic DNA (RAPD) fingerprinting in which a single arbitrary short oligonucleotide is used as a primer to scan a genome for small inverted repeats and to amplify the intervening DNA segments. However, the accuracy and reproducibility of RAPD are still controversial [10]. In order to overcome this difficulty, Hearne et al. [11] and Ishibashi et al. [12] developed CA-repeated primed PCR, although the usefulness of this method for the measurement of relatedness has not yet been examined in animals.

In the present study, we applied CAP-PCR DNA fingerprinting to estimate the mean relatedness and the insemination frequency of females in a small ant species, *Myrmecina graminicola nipponica.* All of the eight developed primers gave variation of band pattern. When analyzed by Primer 5, which resulted in the largest variation, the bands of male origin were always present in all daughters, evidently demonstrating the monandry of this ant species. The mean relatedness was estimated to be 0.47 between mother and daughter and 0.80 between daughters, which was remarkably close to the theoretically expected values of 0.5 and 0.75, respectively. We therefore conclude that CAP-PCR DNA fingerprinting is useful for analyzing relatedness within colonies of small social insects.

*Myrmecina graminicola nipponica* is widespread in Japan and shows functional monogyny; viz. each colony has only one inseminated reproductive

 

Table 1. Number of bands and band-sharing probability in eight primers designed for CAP-PCR. For each primer, four adults of *Myrmecina graminicola nipponica* were examined. Pr. 5 showed the lowest band-sharing probability, i.e., the highest variation of band pattern

| | Pr. 1 | Pr. 2 | Pr. 3 | Pr. 4 | Pr. 5 | Pr. 6 | Pr. 7 | Pr. 8 |
|---|---|---|---|---|---|---|---|---|
| No. of bands | | | | | | | | |
| Range | 16 | 5–7 | 6–7 | 5–6 | 8–11 | 8–11 | 3–8 | 13–19 |
| Mean | 16 | 5.5 | 6.8 | 5.8 | 9.5 | 9.8 | 4.8 | 15.8 |
| Band-sharing probability (%) | | | | | | | | |
| Range | 81.3–100 | 71.4–100 | 83.3–100 | 83.3–100 | 25–75 | 45.5–88.9 | 37.5–100 | 42.1–76.9 |
| Mean | 87.5 | 90.9 | 88.9 | 93.3 | 41.7 | 65 | 73.7 | 57.1 |

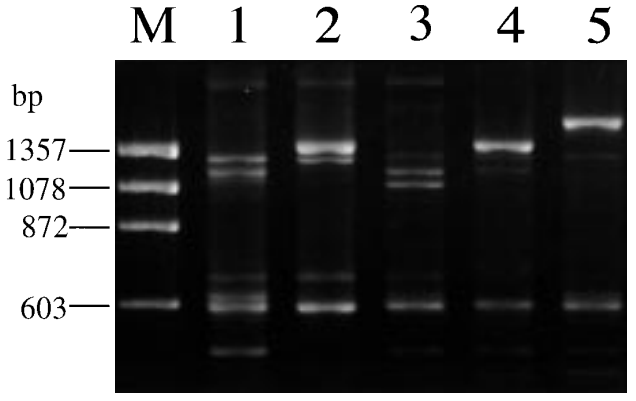


Fig. 1. Intercolonial variation of band pattern induced by Pr.5. M ∅ X174-*Hae*III marker; *1–5* five workers from different colonies

female, i.e., queen or intercaste [13]. In the present study, 12 colonies were collected in a forest near TOEF (Tomakomai Experimental Forest of Hokkaido University) in mid to late August, 1993. In each colony, all adults were crushed with a homogenizer in 250 µl STE buffer. After addition of 10 µl 10% SDS, the homogenate was incubated with 5 µl proteinase K (50 µg/µl) at 37°C for 8–18 h. DNA was extracted twice with phenol (250 µl; equilibrated at pH 8 with TE) and once with 250 µl chloroform-isoamylalcohol (25:1). DNA was precipitated with 2 vol 100% ethanol and 10 µl 5 M NaCl kept at –80°C for 10 min. This precipitated DNA was washed with 70% ethanol and resuspended in TE buffer. The concentration of DNA was measured by spectrophotometer.

The following eight CA-repeat primers were used in PCR: Pr.1: $(CA)_7$-(A/G/T)-G; Pr.2: $(CA)_7$-(A/G/T)-A; Pr.3: $(CA)_7$-(A/G/T)-T; Pr.4: $(CA)_7$-(A/G/T)-C; Pr.5: $(AC)_7$-(G/T/C)-G; Pr.6: $(AC)_7$-(G/T/C)-A; Pr.7: $(AC)_7$-(G/T/C)-T; Pr.8: $(AC)_7$-(G/T/C)-C [12]. Reactions were carried out in 25-µl solution containing 50 ng genomic DNA, 0.5 µM primer, 0.5 unit *Taq* DNA polymerase (Perkin-Elmer). DNA was amplified in a thermal cycler using the following program: after 2 min of initial denaturation at 92°C, a cycle of 30 s denaturation at 91°C, 30 s annealing at 50°C, and 1 min extension at 72°C was replicated 35 times and followed by a final 10-min extension at 72°C. The amplified samples were kept at 4°C before separation on 3% agarose gels. DNA fragments were stained with ethidium bromide and visualized under UV light. This process of reaction was duplicated for each sample and only reproducible bands were adopted in this study.

Prior to the analysis of the 12 colonies, the variation of band patterns was preliminarily tested by using four adults per primer from other colonies. Then, all adults of the 12 colonies were analyzed with a primer which showed the greatest variation of banding patterns in the preliminary test.

In the analysis of four adults by each primer, the mean number of bands was 4.8–19 and the mean band-sharing probability was 0.42–0.93 (Table 1). The lowest band-sharing probability was given by Pr.5 and, therefore, this primer was used for analyzing the mean relatedness. The 12 colonies were composed of 135 adults including 12 inseminated reproductive females. The amount of DNA per individual varied from 94.3 ng to 1683.0 ng (mean ± SD: 354.6 ± 197.5 ng). This variation seemed to depend partly on body size and ovarian development of each adult. Number of bands amplified by CAP-PCR with Pr.5 ranged from 5 to 11 (mean: 7.8). The PCR reaction was duplicated for each sample, and the two band patterns were almost always identical, suggesting high reproducibility of CAP-PCR.

Bands of male origin were always shared by all daughters. This denoted monandry, i.e., each female was inseminated by a single male. Also, the band-sharing probability between colonies was considerably low. For instance, Fig. 1 shows the band patterns of individuals which were chosen from 5 of 12 colonies. The intercolonial band-sharing probability ranged from 0.22 to 0.59 with an average of 0.37 (Table 2).

Band-sharing probabilities were modified into estimated relatedness (r) as follows:

$$r = (w - b)/(1 - b),$$

where w and b are within- and between-probabilities of band-sharing, respectively [14]. As shown in Table 2, mean estimated relatedness was 0.47±SD 0.17 between mother and daughter and 0.80±SD 0.08 between daughters, which were considerably close to the 0.5 and 0.75 theoretically expected under the condition of mon-

Table 2. Modified regression estimates of relatedness for independently identified mother and daughter (M-D), daughter and daughter (D-D) in *Myrmecina graminicola*

| Colony | Band-sharing probability | | | Modified regression estimate r=(w–b)/(1–b) | |
|---|---|---|---|---|---|
| | Within colony (w) | | Between colonies (b) | M-D | D-D |
| | M-D | D-D | | | |
| 1 | 0.45 | 0.93 | 0.32 | 0.21 | 0.90 |
| 2 | 0.63 | 0.91 | 0.48 | 0.29 | 0.83 |
| 3 | 0.64 | 0.82 | 0.22 | 0.53 | 0.77 |
| 4 | 0.71 | 0.87 | 0.40 | 0.52 | 0.78 |
| 5 | 0.66 | 0.86 | 0.39 | 0.44 | 0.77 |
| 6 | 0.75 | 0.96 | 0.59 | 0.39 | 0.90 |
| 7 | 0.66 | 0.77 | 0.26 | 0.54 | 0.69 |
| 8 | 0.79 | 0.86 | 0.46 | 0.61 | 0.74 |
| 9 | 0.87 | 0.96 | 0.22 | 0.83 | 0.95 |
| 10 | 0.70 | 0.85 | 0.33 | 0.55 | 0.78 |
| 11 | 0.60 | 0.86 | 0.46 | 0.26 | 0.74 |
| 12 | 0.65 | 0.84 | 0.35 | 0.46 | 0.75 |
| Mean ± SD | 0.68 ± 0.10 | 0.87 ± 0.06 | 0.37 ± 0.11 | | |
| Estimate of relatedness (mean ± SD) | | | | 0.47 ± 0.17 | 0.80 ± 0.08 |

andry. The present study obviously showed that CAP-PCR DNA fingerprinting has the following advantages for analyzing kin relatedness. (1) The required amount of DNA is as small as 50 ng, which is available even from very tiny insects. (2) The variation of band pattern is large and only a few individuals or colonies are required for the analysis of kin relatedness. (3) Band patterns from the same sample are almost always identical; thus, the reproducibility is very high.

The primers already developed for CAP-PCR are known to indicate the variation of band pattern in some animals including human, mouse, Blakiston's fish owl, short-tailed bush warbler, Manchurian crane, masu salmon, and gall-making aphids [12]. This suggests that the CAP-PCR DNA fingerprinting is extensively useful for analyzing relatedness in a variety of animal species.

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