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Research article

Semi-nondestructive genetic sampling from live eusocial wasps, *Polistes dominulus* and *Polistes fuscatus*

P. T. Starks[1] and J. M. Peters[2]

[1] *Division of Insect Biology, University of California, Berkeley, CA 94720-3112, USA, e-mail: starks_p@uclink.berkeley.edu*
[2] *University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235, USA*

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Summary. We present a method of extracting DNA from medium-sized paper wasps without sacrificing the animal. From the distal half of a single leg, enough DNA is extracted for 125 PCR reactions. This DNA – collected from *Polistes dominulus* and *P. fuscatus* – amplifies as well as DNA extracted from larger amounts of tissue taken from sacrificed animals, and the resulting microsatellite regions amplify faithfully. Although the nest tenure of *P. dominulus* workers with leg-cuts was shorter than control animals, test animals appeared to behave normally. This DNA extraction method is well suited for studies that combine behavioral observations and genetic analysis on animals likely to disappear during the observation period. Collecting genetic data without destroying colonies will enable researchers to examine behavior in eusocial insects without damaging the future population size or altering the population genetic structure.

Key words: Behavior, DNA extraction, DNA fingerprinting, genetic analysis, PCR.

Introduction

Genetic analysis has had a profound impact on the studies of nepotism in eusocial insects, especially in *Polistes* wasps (Strassmann, 1996). Unfortunately, DNA extraction methods usually call for grinding the whole insect or large and essential sections of the insect (e.g., the thorax or head capsule; Strassmann et al., 1996; see also Phillips and Simon, 1995). Since dead insects do not behave in ways meaningful to behavioral researchers, all behavioral observations must be performed prior to genetic analysis. Additionally, since genetic analysis commonly calls for the sacrifice of the insect, aggressive sampling from a population may diminish the subsequent population size and alter the population genetic structure.

We attempted to sample from populations of *Polistes dominulus* and *P. fuscatus* without sacrificing the subjects. A distal portion of a single leg was collected from test wasps and DNA was recovered from this segment using a modification of the Peters et al. (1995) sperm DNA extraction method. To ensure consistency of microsatellite amplification, leg-cut samples were compared with DNA extracted from the thoracic muscle of sacrificed *P. fuscatus* workers. In addition, *P. dominulus* colonies containing leg-cut subjects were videotaped to determine the effect of this procedure on worker survivorship and behavior.

Materials and methods

General methods

In July and August 1995, ten post worker-emergence colonies of *Polistes dominulus* were located at two field sites in Ithaca, NY (colony size; $\bar{x} \pm se$ 13.10 ± 1.77 workers). On the mornings of July 20, 22, 29 and August 8, two to three colonies of test animals were briefly anesthetized with ether, individual wasps were censused and given unique thoracic marks with Testors enamel paint, and leg segments were taken from approximately 33% of each colony's worker population (N = 10 colonies; N = 43 total leg-cut samples). Worker wasps were selected at random for surgery. In May of 1996, 37 pre worker-emergence multiple-foundress colonies of *P. fuscatus* (N = 121 wasps) were located at six field sites in Ithaca, NY. On the mornings of May 23–28 foundresses were removed from their nest, given thoracic marks, subjected to leg-cuts and then returned to their nests. *P. fuscatus* foundresses were not anesthetized with ether prior to surgery but rather were chilled in a field cooler.

In both 1995 and 1996, a single leg from wasps selected for surgery was severed just distal to the joint between the femur and tibia using fingernail clippers cleaned with alcohol and water between collections. An attempt was made to collect only mesothoracic legs. Due to wasp movement, however, we were not completely successful and a few prothoracic and metathoracic legs were collected. (A maximum of one leg was taken per individual.) Leg segments were stored in labeled 1.5 ml Eppendorf tubes at –20 °C. Additional *P. fuscatus* workers (N = 12) were collected in August 1996 and used to compare leg and thorax extraction

methods. Three days after legs were removed, the 1995 *P. dominulus* colonies were videotaped for two hours. Surgery did not appear to be fatal to the colony since all of the *P. dominulus* colonies completed the normal colony cycle and thus survived to produce reproductives.

Laboratory methods

With each collected sample we, respectively: (1) ground the lower leg using a disposable minipestle (Strassmann et al., 1996), (2) added 25 µl of 50 mM DTT (Dithiothreitol), (3) added 25 µl of 0.5 M KOH, (4) spun for 5 s at 13,000 rpm, (5) incubated in a heat block for 5 min at 65 °C, (6) vortexed for 10 s, (7) incubated in a heat block for 5 min at 65°C, (8) spun for 10 s at 13,000 rpm, (9) added 25 µl of 0.5 M HCl, (10) added 8.4 µl of 0.5M Tris-HCl pH. 9.0, (11) spun for 10 s at 13,000 rpm, (12) mixed by hand agitation, (13) spun for 1 min at 13,000 rpm, and (14) transferred 50 µl of the supernatant into a labeled 500 µl tube containing 200 µl of milli-Q water. (Note: The 0.5 M KOH and 0.5 M HCl must combine to produce a 7.0 pH mixture.) The diluted supernatants were used for PCR and these PCR products were visualized as in Peters et al. (1995). Detailed amplification and visualization protocols are given elsewhere (Peters et al., 1995; Strassmann et al., 1996).

Statistical methods

Microsatellite amplification success rate was calculated from the 1996 *P. fuscatus* foundress samples (N = 121). Amplification accuracy was visually examined by comparing separate samples taken from *P. fuscatus* workers: a leg-cut extraction and a thorax extraction from the same animal. Since identification of individual thoracic paint marks is easily made via videotape, *P. dominulus* worker tenure was determined via census data gathered from videotape. Each cohort of *P. dominulus* workers (those marked on the same day) contained individuals with leg-cuts (N = 43) and without leg-cuts (N = 88). Individuals were scored as either present or absent during the two hour videotape period taken three days after leg removal. Videotapes were taken between approximately 11 AM and 4 PM during periods of high worker activity. A paired *t*-test test was used to compare the percent of workers with leg-cuts and the percent of workers without leg-cuts that were present on their colonies at any time during the videotaped period. We do not anticipate that time of day will impact our general conclusions since each comparison is internally controlled (i.e., each colony contained both classes of wasp).

Results

113 of 121 *Polistes fuscatus* foundress samples successfully amplified (93.4%). No difference in microsatellite amplification was observed when comparing leg-cut samples with dilute thorax preps taken from sacrificed *P. fuscatus* workers (i.e., microsatellite length was not altered by the extraction method). *P. dominulus* samples amplified well and were used to screen primers for future research. *P. dominulus* worker tenure, as measured by the percent of individuals observed on their colony during the two-hour census period, was shorter for wasps subjected to the leg-cut procedure (62.79 ± 6.12%) than for controls (80.68 ± 1.82%; paired *t*-test: $t_9 = 2.92$, p = 0.017). Although fine-scale analysis of worker behavior was not undertaken, workers with leg-cuts that were present on the nest during the two-hour census period were observed to perform normal colony tasks (e.g., sharing foraged items, feeding larvae, etc.).

Discussion

The leg-cut procedure is effective both in terms of amplification success and scoring reliability. The procedure, however, may be detrimental to some animals. Although the apparent survival of leg-cut recipients was lower than the controls, those that remained performed tasks necessary for colony survival. Animals that disappeared may not have perished but may have abandoned the nest to pursue alternative reproductive options such as entering early diapause (Reeve et al., 1998; Starks, 2001) or founding satellite nests (Strassmann, 1981). Alternatively, leg-cut subjects may not have abandoned the colony but rather spent a disproportionate amount of time performing tasks away from the colony (e.g., foraging). In addition, some leg-cut individuals may have been forced from the colony by the remaining nestmates. Regardless, since the leg-cut procedure leads to a decrease in nest tenure – and possibly an increase in worker mortality – this procedure is most suited for use on sterile workers from small to medium sized eusocial insect colonies, i.e., those colonies that can afford to lose slightly more individuals than normal but cannot afford to donate workers entirely.

This procedure will allow researchers to collect genetic samples from all individuals on a colony while decreasing colony strength by only ~17.5%. For the same amount of information, traditional methods of genetic sampling would necessitate the destruction of the colony. In colonies with multiple reproductively active females, the leg-cut procedure allows for repeated sampling as new workers eclose, thus enabling studies quantifying the degree of nepotistic behavior as a function of time. Due to the effect of time on reproductive skew (Reeve et al., 2000) and on normal worker mortality (see Results), waiting until the end of a field season to collect animals for destructive genetic sampling may lead to biased estimates of population genetic structure. By collecting genetic data without destroying the colony, researchers can more precisely examine the adaptive significance of behavior in eusocial insects without significantly damaging future populations.

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