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BIOGEOGRAPHY OF BAHAMIAN *CAMPSOMERIS* (Hymenoptera: Scoliidae)

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ABSTRACT

Three morphologically similar Bahamian scoliid wasps have traditionally been placed in three taxa as follows: two species, *Campsomeris bahamensis* and *C. trifasciata*, the latter of which is divided into two subspecies: *C. t. trifasciata* and *C. t. nassauensis*. However, the variation among these groups may be no greater than that found within the same taxon collected from different islands. In order to test this hypothesis, we compared the genetic diversity of different *Campsomeris* populations, collected at various locations on several islands. DNA was extracted from individual male and female specimens. Replicate portions of the DNA were preferentially amplified by the polymerase chain reaction, using a unique random primer for each analysis. The resultant DNA products were compared by electrophoresis. Preliminary results indicate overlapping genetic profiles between these taxonomic groups.

BACKGROUND

Wasps of the genus *Campsomeris* are widely distributed throughout the Bahamian archipelago. Three morphologically similar taxa have been described. One is the species *Campsomeris bahamensis* (Bradley 1928), which occurs in the southern Bahamas, and which we have collected from Acklins and Great Inagua Islands. The second species, *Campsomeris trifasciata*, is further divided into two subspecies: *C. trifasciata trifasciata*, which occurs throughout much of the West Indies, including the islands of Cuba, Jamaica, Haiti and Puerto Rico; and *C. trifasciata nassauensis*, from the northern and central Bahamas (Bradley 1928). Bradley described *C. t. nassauensis* from the northern and central Bahamas in 1928, but *C. t. trifasciata* occurs on some of the southeastern Bahamian islands including San Salvador, and Mayaguana. In fact, some of these Bahamian specimens, identified as *C. t. trifasciata* by Bradley, occur in museum collections at the American Museum of Natural History (Elliott, pers. observation). Specimens with characteristics of both *C. t. trifasciata* and *C. bahamensis* occur on Acklins Island (Elliott, pers. observation).

While the haploid males from all three taxa are virtually indistinguishable, Bradley (1928, 1964) differentiated them on the basis of the characteristics of the females. Females of *C. trifasciata trifasciata* have broad yellow/gold abdominal bands; those of *C. trifasciata nassauensis* have broad black abdominal bands alternating with narrow yellow ones. Females of *C. bahamensis* have broad yellow/gold abdominal bands and red pubescence on their heads (Bradley 1964). However, the overlapping distribution of these taxa within the Bahamian islands suggests that these minor morphological differences may not correlate with distinct genetic profiles. It is possible that the normal genetic variation found within one taxon may be equal to the variation found among these taxa. In other words, the three taxa as described may actually represent genetic variants of a single taxon.

The genetic diversity of these species was investigated using the technique of random amplified polymorphic DNA (RAPD) (Williams *et al.* 1990, Welsh 1990). This technique is based upon the polymerase chain reaction (PCR), and has been used to study the genetic diversity or paternity of several organisms, including grasshoppers (Chapco 1992; Kosal 1998), honey bees (Hunt 1992), burying beetles (Scott 1993) and mice (Welsh 1991). A short DNA oligonucleotide primer of arbitrary sequence is used for the targeted replication of

several DNA sequences within the DNA of individual wasps. The resultant DNA bands are then analyzed. Two wasps with identical banding patterns are probably members of the same species. Two wasps with very few differences in their banding patterns may also be members of the same species. The greater the differences in banding patterns, the more distant the species relationship. Definitive results can be generated only after several analyses, each with unique primers.

MATERIALS AND METHODS

Template DNA Isolation

Wasps were placed in ethanol immediately upon capture. Flight muscle DNA was isolated by the protocol previously described for grasshopper leg muscle DNA (Kosal 1998). Thoraxes were individually minced, mixed with digestion buffer (1 mg/mL proteinase K, 5 % SDS, 100 mM NaCl, 50 mM Tris (pH 7.5), 1 mM EDTA) and incubated at 55 °C for 1 hr. The samples were extracted once with phenol:chloroform:isoamyl alcohol (p:c:ia; 25:24:1 by volume), then incubated with 40 µg/µL RNAse A at 37 °C for 15 min. The DNA was extracted three times with p:c:ia and quantitated by absorbance at 260 nm. The DNA yields from each wasp ranged from 180 - 2,130 ng.

DNA Analysis

Two ng (low concentration) or twenty ng (high concentration) of template DNA were mixed with 1 unit *Taq* polymerase and 2 µM primer (GAGCAATTCGCCTCGAT) in 5X buffer (300 mM Tris-HCl, 75 mM ammonium sulfate, 12.5 mM MgCl2, 2.5 mM each dNTP, pH 8.5). Four low stringency amplification cycles (94 °C x 1 min. → 40°C x 1 min. → 72 °C x 2 min.) were followed by 40 high stringency cycles (94 °C x 1 min. → 60°C x 1 min. → 72 °C x 2 min.), as described by Welsh (1995). Sixteen microliters of PCR product were separated on a 1.7 % agarose gel in Tris/acetate/EDTA running buffer. The gels were stained in 0.5 µg/mL ethidium bromide and photographed. The samples were randomized prior to PCR and identified following gel separation.

RESULTS

The results of the DNA amplification are shown in Figure 1. Each sample pair (low and high concentrations of DNA) represents an individual wasp collected from the indicated island. On some islands, only males were collected (Andros, Grand Bahama, Mayaguana, and San Salvador).

Two bands were present in all samples: 560 bp and 870/890 bp (see also Table 2). Due to resolution differences between the gels in Figure 1, the 870/890 bp band cannot be sized accurately. One band was present in all but one sample (790 bp); the Grand Bahama male showed a 740 bp band instead. Wasps from eight islands have a 1600 bp band in the male and/or female. In addition, two male samples have an additional high molecular weight band (1070 bp, Acklins; 1373 bp, San Salvador; Table 3). All of these high molecular weight bands were produced only in the samples with the low concentration of template DNA.

CONCLUSIONS

The banding patterns of the three taxa are very similar, as expected. All specimen share two bands (560 bp, 870/890 bp), and several specimens share two other bands (790 bp, 1600 bp). However, two of the three taxa analyzed demonstrate intraspecies genetic diversity.

C. trifasciata nassauensis has three genetic groups. One group (Andros, N. Providence, Eleuthera, and Long; northern and central islands) show the same general banding pattern, although there are some differences between male and female specimens collected from the same island. The second (Grand Bahama; northernmost island) and third (Cat; central island) groups each have a unique banding pattern. The finding of a unique banding pattern in *C. t. nassauensis* from Grand Bahama is interesting in that it corresponds to a significant difference in morphological variationbetween specimens of *C. t. nassauensis* from Grand Bahama and those from all the islands ofthe Great Bahama Bank reported by Elliott and Elliott at the Fifth Symposium on Natural History of the Bahamas (unpublished).

Table 2. Banding patterns of PCR products derived from the low concentration of template DNA (2 ng). M, male; F, female. ?, band not clearly present or absent on the gel (See Figure 1). _, absence of a band in one sex. *, only high concentration of template DNA analyzed (20 ng).

	DNA band of particular length (basepairs)							
Specimens	250	560	740	790	870/890	1070	1375	1600
C. bahamensis								
Great Inagua (M)		x		x	x			x
" " " " (F)		x		x	x			?x
C. trifasciata trifasciata								
Acklins (M)	x	x		x	x	x		x
Acklins (F)	-	x		x	x	-		x
Mayaguana (M) *		x		x	x			
San Salvador (M)		x		x	x		x	x
C. trifasciata nassauensis								
New Providence (M)		x		x	x			-
New Providence (F)		x		x	x			x
Cat (M)	-	x		x	x			?x
Cat (F)	x	x		x	x			?x
Long (M)		x		x	x			x
Long (F)		x		x	x			-
Eleuthera (M)		x		x	x			x
Eleuthera (F)		x		x	x			x
Andros (M)		x		x	x			x
Grand Bahama (M)		x	x		x			?x

Table 3. PCR products that appear in only one or two wasps.

Species/Locations	Unique Bands	Rare Bands
C. trifasciata trifasciata		
Acklins (only in M)	1070 bp	250 bp
San Salvador (only M analyzed)	1375 bp	
C. trifasciata nassauensis		
Grand Bahama (only M analyzed)	740 bp	
Cat (only in F)		250 bp

C. trifasciata trifasciata has at least two genetic groups. Both Acklins (southern island) and San Salvador (eastern island) have unique banding patterns, each with a different high molecular weight DNA band. The genetic placement of the male from Mayaguana (eastern island) is uncertain, since the high molecular weight bands were not uniformly produced in this analysis. However, since the Mayaguana male is also missing the lowest molecular band, it may be most genetically similar to the San Salvador male.

C. bahamensis was collected on only one island (Great Inagua, southernmost island). However, it shares the DNA banding pattern seen in the largest genetic group from *C. trifasciata nassauensis*, found on most of the central islands.

The morphological differences between the three taxa of *Campsomeris* are not supported by distinct genetic profiles in this analysis. One species (*C. trifasciata*) has as much genetic diversity within each subspecies as it does between the subspecies. The second species (*C. bahamensis*) does not have a unique genetic composition. In addition, the three taxa have overlapping geographic distributions. These preliminary results support the hypothesis that the genetic variation among these taxa is not greater than the variation within one taxon. This points to the possibility that the three taxa may in fact be diverse genetic populations within one taxon.

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FIGURE 1A

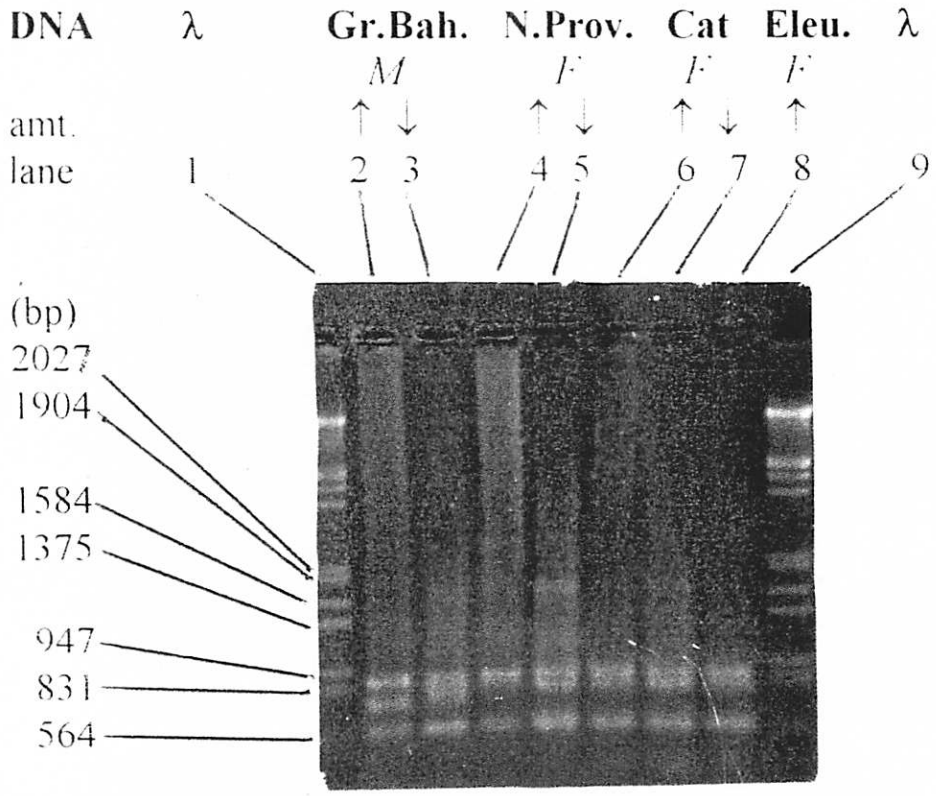

FIGURE 1B

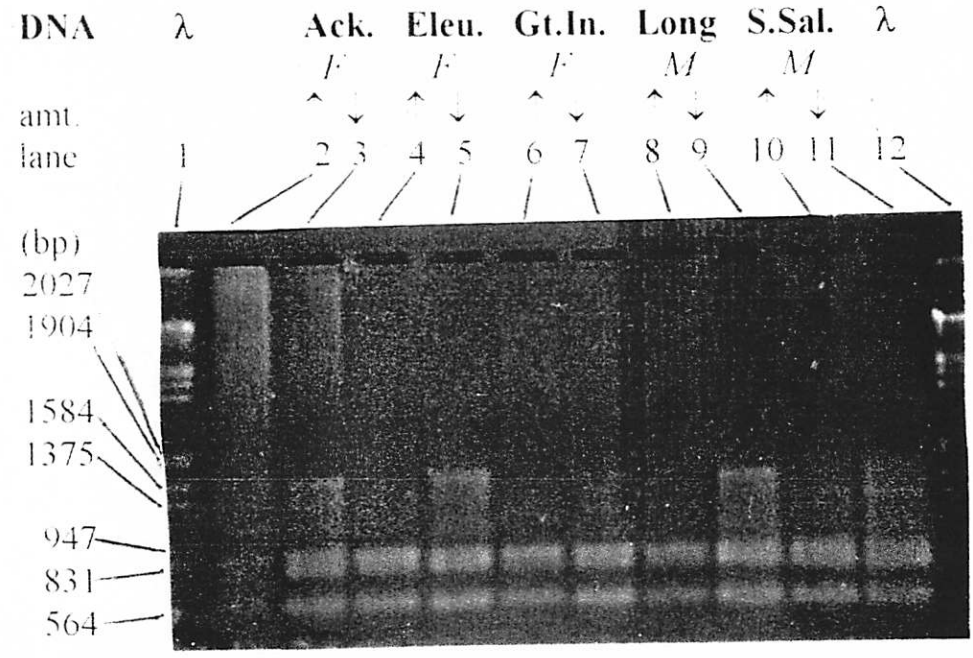

FIGURE 1C

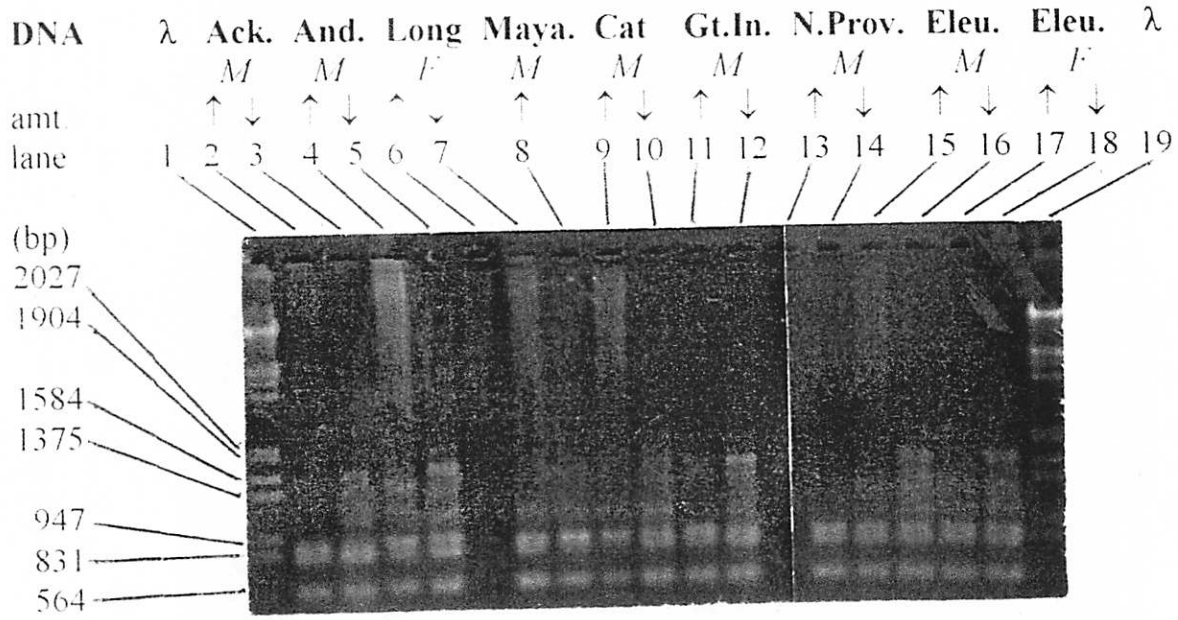

FIGURE 1. Agarose gel electrophoresis of PCR products. Different amounts of template DNA (20 ng, ↑; 2 ng, ↓) were amplified per wasp. λ (lambda DNA), molecular weight marker. *M*, male; *F*, female; *bp*, base pair; *Ack.*, Acklands; *And.*, Andros; *Cat*, Cat; *Eleu.*, Eleuthera; *Gr.Bah.*, Grand Bahama; *Gt.In.*, Great Inagua; *Long*, Long; *Maya.*, Mayaguana; *N.Prov.*, New Providence; *S.San.*, San Salvador. PCR products from the high concentration of female Eluethera template DNA were included on each gel (*A, B, C*) for direct comparison of the banding pattern.