

ELECTROPHORETIC AND ANTIGENIC COMPARISONS
OF THE IXODID TICKS *AMBLYOMMA MACULATUM*
AND *A. AMERICANUM* (ACARI: IXODIDAE)

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ABSTRACT

Disc gel electrophoretic patterns of female and male *Amblyomma maculatum* Koch extracts are similar but not identical. A comparison of combined male-female *A. americanum* (L.) with male and female *A. maculatum* extracts suggests that several common proteins exist. Sera from rabbits immunized with unfed male *A. maculatum* extract formed a precipitin band with both male and female *A. maculatum* antigens as well as the combined male-female *A. americanum* antigens. However, sera from calves immunized with unfed male and female *A. maculatum* gave no detectable precipitin reactions.

RESUMEN

Los patrones electrofóricos formados en discos de gelatina por los extractos de hembras y machos de *Amblyomma maculatum* Koch son similares, pero no idénticos. Una comparación del extracto combinado de los machos-hembras de *A. americanum* (L.), y el de machos y hembras de *A. maculatum*, sugiere que estos tienen varias proteínas en común. Los sueros de conejos, inmunizados con extracto de machos *A. maculatum* sin alimentar, formaron una banda precipitante con antígenos de ambos machos y hembras de *A. maculatum* y *A. americanum*. Sin embargo, los sueros de terneros, inmuizados con machos y hembras de *A. maculatum* sin alimentar, no demostraron ninguna reacción precipitante detectible.

Numerous studies have shown that animals develop antibodies to tick infestation or immunization with tick extracts (Riek 1959, Wikel and Allen 1976, Allen and Humphreys 1979, and McGowan et al. 1980). These antibodies are thought to be one component of acquired resistance to ticks (Wikel and Allen 1976).

Trager (1939) observed that guinea pigs first infested with either *Dermacentor andersoni* Stiles or *D. variabilis* (Say) exhibited a cross resistance to larvae of the other species. Similar results were observed in rabbits infested with *D. variabilis* and *Haemaphysalis leporispalustris* (Packard) (Trager 1939, McGowan et al. 1982a). Cross-resistance in guinea pigs to *D. andersoni*, *D. variabilis*, *Amblyomma americanum* (L.) and *Ixodes scapularis* Say was studied by McTier et al. (1981). They concluded that guinea pigs resistant to *D. andersoni* were also resistant to *D. variabilis* but not to *A. americanum* or *I. scapularis*. Furthermore, guinea pigs resistant to *A. americanum* were resistant to *D. variabilis* but not to *D. andersoni*. These

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findings suggest that antigens may be shared among some tick species. In the present study extracts of *A. maculatum* Koch and *A. americanum* were examined by polyacrylamide gel electrophoresis and with the combined polyacrylamide gel-agar gel immunodiffusion technique for the presence of common antigens.

MATERIALS AND METHODS

Preparation of unfed tick extracts and polyacrylamide disc gel electrophoresis were as outlined by McGowan et al. (1980). Briefly, the tick extracts were electrophoresed in 7.5% gels with a pH of 8.9 and an electric current of 2mA/tube. Gels were stained for the presence of proteins with analine blue-black. A Beckman DU spectrophotometer with a Gilford Model 2410 scanning attachment was used to scan stained gels at 550 nm and a slit width of 0.2 mm.

Four New Zealand White rabbits of both sexes that weighed 1.8-2.2 kg, were injected in the footpads with 3 mg/kg of unfed male *A. maculatum* antigen dissolved in sterile water and mixed with an equal volume of Freund's complete adjuvant (FCA). A subsequent injection of antigen in Freund's incomplete adjuvant (FIA) was administered 14 days following the initial injection. Hereford bull calves that weighed ca. 215 kg were injected with 6 mg/kg of combined male-female *A. americanum* antigen in FIA at 2 week intervals for a total of three injections. Serum for serological tests was collected prior to immunization and at weekly intervals during the study.

The basic procedure for the combined polyacrylamide gel-agar gel immunodiffusion was modified from that of Wright et al. (1971). Following disc gel electrophoresis the unstained gels were extruded, placed on glass plates (100 x 100 x 3 mm) and overspread with 1% agar (Ionagar #2, Colab Laboratories, Inc., Chicago Heights, IL) in phosphate buffered saline. The plates were incubated at room temperatures (23-25° C) for 24 h to allow diffusion of antigens into the agar. Troughs (2 x 85 mm) were then cut 5-8 mm from and parallel to the disc gels, charged with serum, and the plates incubated at room temperature for 24-48 h.

RESULTS

The electrophoretic patterns of female and male *A. maculatum* are similar but not identical (Fig. 1A and B). There appears to be at least one additional protein and increased concentrations of several common proteins in the male extract (Fig. 1A). A comparison of the combined male-female *A. americanum* (Fig. 1C) with male and female *A. maculatum* (Fig. 1A and B) suggests that several common proteins exist, as seen in the top 5 to 7 bands. There are, however, distinct species differences as evidenced by the difference in patterns toward the bottom of the gels. The sensitivity of the scanner was set automatically thereby causing the anode peak to appear to be of the same magnitude in all 3 scans.

Sera from rabbits immunized with male *A. maculatum* extract formed a single precipitin band with both male and female *A. maculatum* antigens (Fig. 2A) as well as the combined male-female *A. americanum* antigens (Fig. 2B).

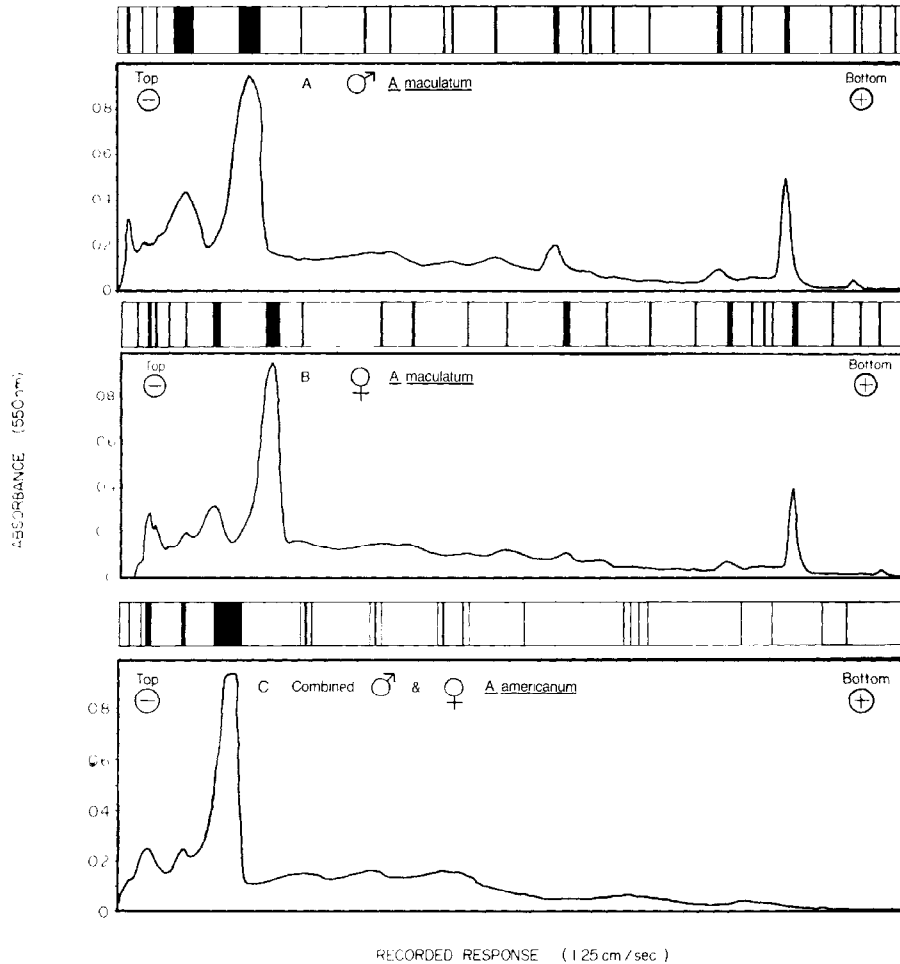


Fig. 1. Disc polyacrylamide gel (7.5%, pH 8.9, 2mA/tube) electrophoresis of unfed whole adult tick extracts. A. Male *Amblyomma maculatum* scan. B. Female *A. maculatum* scan. C. Combined male-female *A. americanum* scan.

No detectable reactions were observed with sera from nonimmunized rabbits or immunized calves.

DISCUSSION

Previous studies utilizing electrophoretic techniques have demonstrated that tick extracts contain many proteins, some of which are shared among species of a genus. Krasnobaeva et al. (1971) reported similarities of the electrophoretic patterns on agar gel of 3 species of *Hyalomma* ticks. Similarities were also observed between the genera *Hyalomma* and *Dermacentor*.

Similar findings were noted in the present study of *A. maculatum* and *A. americanum* utilizing the more sensitive technique of polyacrylamide gel electrophoresis. Distinct differences were observed in the electrophoretic pat-

terns of these two species, however, the 5 to 7 bands near the top of the gels were similar in appearance (Fig. 1A, B and C). Differences in the electrophoretic pattern between male and female *A. maculatum* were seen. The male extract appears to contain at least one additional band as well as higher concentrations of several proteins. Comparison of these banding patterns suggests but does not prove the existence of common proteins between male and female *A. maculatum* and between *A. maculatum* and *A. americanum*.

Prior studies have shown that animals immunized with tick extracts develop precipitating antibodies. Using gel diffusion Riek (1959) found that sera from rabbits immunized with extracts made from the eggs and larvae of *Boophilus microplus* (Canestrini) formed 1-7 distinct precipitin bands when tested against the extracts. Similar findings were reported by Boese (1969, 1974) who observed precipitating antibodies in rabbits injected with whole-tick extracts of *H. leporispalustris*. Precipitating antibody was also observed in cattle immunized with extracts of *D. andersoni* (Allen and Humphreys 1979).

Only a single male *A. maculatum* protein located near the top of the disc gel reacted with antibodies in the sera from injected rabbits. We are uncertain as to the reason for this, however, based upon gel scans (Fig. 1) and precipitin patterns (Fig. 2), the immunogenic protein appeared to be present in the largest quantity. Other proteins in the extracts may have induced antibody production had they been present in sufficient quantities. Allen and Humphreys (1979) observed no response in cattle to injection of extracts of unfed ticks but reported circulating antibody production in cattle immunized with ticks which had been fed for five days prior to antigen preparation. They hypothesized that feeding increased gut enzyme concentrations in tick extracts which may increase the extracts' antigenicity. This increase in the number and/or concentration of tick proteins was seen in *A. americanum* which had fed for 5 days prior to extract preparation (McGowan et al. 1982b). Our findings support these observations in cattle, but the unfed tick extract did cause resistance in rabbits (McGowan et al. 1980).

The cross reactivity of the *A. maculatum* antisera with the *A. americanum* antigens suggests that at least one of the proteins which appears to be similar in the disc gels also shares antigenic determinants. The *in vivo* studies of Trager (1939), McTier et al. (1981) and McGowan et al. (1982a) suggest that the resistance which develops following repeated infestations extends to other members of the same tick species. Although the present study does not determine the *in vivo* effect of immunization with these extracts one may speculate that these or other unidentified proteins may induce or enhance host resistance to tick infestations.

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THE IMPORTANCE OF BEES TO KAYAPÓ INDIANS OF THE BRAZILIAN AMAZON

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ABSTRACT

A total of 56 folk species of *Apidae* are discussed in the classification system of the Kayapó Indians of the Brazilian Amazon; 54 of these are stingless Meliponidae. These folk species correspond to 66 scientifically recognized species, reflecting an 86-percent correlation between scientific and folk taxonomic systems. A highly specialized indigenous knowledge about bee behavior (folk ethology) exists that allows for the semi-domestication of 9 folk species. Folk ethology is a field little appreciated by Western science, yet Kayapó knowledge of bees is evidence that significant information about nature and human-environmental relationships can be gained from analysis of folk taxonomic systems.