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Homology among the autosomal chromosomes of *Boophilus annulatus* (Say) and *B. microplus* (Canestrini)

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HOMOLOGY AMONG THE AUTOSOMAL CHROMOSOMES OF *BOOPHILUS ANNULATUS* (SAY) AND *B. MICROPLUS* (CANESTRINI)

A Thesis

by

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HOMOLOGY AMONG THE AUTOSOMAL CHROMOSOMES

OF *BOOPHILUS ANNULATUS* (SAY) AND

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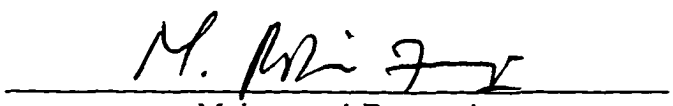

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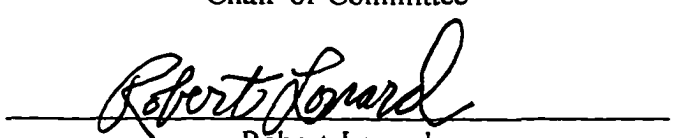

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Autosomes of *Boophilus annulatus* and *B. microplus* were compared using C- and G-bands to establish the degree of homology. Karyotypes of both species consisted of 20 autosomes and an XX:XO sex determination system with the X being the largest chromosome. All chromosomes of *B. annulatus* were acrocentric with heterochromatin limited to centromeric regions. The *B. microplus* karyotype was acrocentric with a single band of noncentromeric heterochromatin occuring in three chromosome pairs. Interspecific comparisons indicated seven pairs of G-band homologous autosomes and three G-band homologous pairs when added interstitial heterochromatin was considered. The number three chromosome of both species showed variation in length at an area of extreme constriction associated with the nucleolar organizer region.

DEDICATION

This work is dedicated with love to my husband, Scott J. Gunn, for always believing in me and to my daughter, Veronica F. Gunn, who has taught me a great deal about what is really important in life.

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I am grateful to my committee members Dr. Mohammed Farooqui (Chairman), Dr. Robert Lonard and Dr. Dan Murray for the time and effort they have extended in support of my education. I am also grateful to Dr. Norman Savage who served as chairman of my committee until his retirement. I thank Dr. Scott Gunn for providing financial support for my research under his NIH grant and for allowing me to continue research in his lab after I was no longer employed on said grant. Dr. Terry Allison was also very gracious in lending me numerous books and reference materials. I thank Dr. Frank Judd for his constructive criticism on papers I wrote in his classes which helped me develop better writing skills.

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This study could not have been completed without the help of other students. I thank the following students for their assistance in dissection of hundreds of ticks used in this study: Marianita Escamilla, Maria Valdez, Enrico Garza, Monica Gonzalez, Jose Gonzalez and George Blackstone. I also thank Maria Teresa Tijerina, Sandy Sefcik and Dana Gonzalez for their moral support.

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INTRODUCTION

Boophilus microplus and *Boophilus annulatus* are important veterinary and economic ectoparasites that act as vectors for many diseases. These species cause irritation of cattle, blood loss, interrupted feeding, hide damage, and act as vectors of *Babesia bigemina*, the protozoan which causes bovine babesiosis (cattle tick fever) (Newton et al., 1972a). The distribution of *B. annulatus* in 1905 covered, in whole or part, 14 of the southern United States (approximately 700,000 square miles) and had significant economic impact on cattle producers in those areas (Graham and Hourrigan, 1977). In 1906, the estimated economic loss attributed to *B. annulatus* was $130,500,000 per year (James and Harwood, 1969). *Boophilus microplus* distribution was more restricted and occupied only southern regions of Texas and Florida (Graham and Hourrigan, 1977).

Efforts to control *Boophilus* ticks began in 1889 when northward movement of cattle from infested areas was restricted by the United States Department of Agriculture. Eradication programs using various chemicals in combination with quarantine procedures have successfully eliminated *Boophilus* ticks from the United States. Since 1948, only rare, small outbreaks of *Boophilus* ticks have occurred due to movement of wildlife and livestock (Graham and Hourrigan, 1977). Some

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This thesis follows the style and format of the journal *Evolution*.

chemical agents used in eradication programs include arsenic, oil emulsions, DDT, and gamma benzene hexachloride. These agents are no longer used due to concerns such as residuals in meat, environmental damage, and acquired chemical resistance of *Boophilus* ticks to chlorinated hydrocarbon insecticides. Companies are reluctant to develop new pesticides or to have existing pesticides relabeled for use in treating cattle due to the expensive and lengthy process of Food and Drug Administration approval.

Coumaphos, an organophosphorus compound introduced in 1968, is currently the chemical agent of choice for controlling *Boophilus* tick infestation and is used in systematic dipping of infested cattle to the near exclusion of all other chemical agents (Davey, 1995). Organophosphorus compounds are neurotoxins which cause hyperexcitation of the nervous system by interfering with the function of acetylcholinesterase. Failure of acetylcholinesterase function leads to a build up of acetylcholine resulting in paralysis and eventually death (Bull and Ahrens, 1988). After cattle are dipped in coumaphos, pastures occupied by infested cattle are vacationed long enough for ticks to die out naturally due to environmental stress and lack of hosts. The time necessary for natural die off varies seasonally due to climate. Pastures need to be vacationed for less time in hot summer months than in cooler winter months (Davey, 1995).

Boophilus ticks are developing resistance to coumaphos creating a need for alternative methods of treating infested cattle (Harris et al., 1988). One alternative method currently being tested involves injecting cattle with a controlled-release form

of ivermectin (Miller, J. A., pers. comm.). Ivermectin is a derivative of avermectin which is isolated from the fungus *Streptomyces avermitilis*. Both of these agents block chloride channels and cause hyperexcitation in the central nervous system by interfering with the action of g-aminobutyric acid (GABA), a synaptic inhibitor. This hyperexcitation results in paralysis and death (Campbell, 1981). Ivermectin has been used to control internal and external parasites such as nematodes, lice, mites, and ticks on many vertebrate hosts (Hotson, 1982). Advantages of this method of tick control include no vacating of pastures, no dipping of cattle, and achievement of complete control in a single treatment (Miller, J. A., pers. comm.)

Currently, the main objective in control of *Boophilus* ticks is prevention of reinfestation. Reinfestation is a constant threat due to both the presence of *Boophilus* ticks in the states of northern Mexico and importation of cattle from infested areas. A 1/4 to 10 mile wide buffer zone (Tick Eradication Quarantine Area) was established in 1943 along the Rio Grande from Brownsville to Del Rio to prevent movement of ticks into the United States. This zone is closely monitored and livestock moving within or across this area are inspected for the presence of *Boophilus* ticks by the Animal and Plant Health Inspection Service, USDA. Due to the difficulty of inspecting large numbers of cattle and free movement of wildlife across this zone, reinfestation is still a constant threat (Hilburn et al., 1989). Reinfestation caused nineteen premises in the United States to be under an USDA infestation quarantine in October of 1996 (Bowers, 1996). Five of these premises were outside of the Tick Eradication Quarantine Area. This quarantine will be maintained and all cattle on

these premises will be treated until eradication of the introduced *Boophilus* tick population is achieved.

Transmission of *Babesia bigemina*, the causative agent of Cattle Tick Fever, is the greatest hazard of *Boophilus* infestation. Effects of *B. bigemina* vary depending on the age of cattle when first exposed. Cattle exposed as calves aquire passive immunity from colostrum of their mother's milk. These calves suffer only mild symptoms and then become carriers of *B. bigemina*. Cattle having first exposure as adults suffer severe symptoms including pneumonia, digestive disorders, encephalitis and high fever which often results in death (Siegmond, 1973). Reintroduction of *Boophilus* ticks to eradicated areas and introduction of susceptible cattle to infested areas have devastating effects because the cattle lack immunity (Graham and Hourrigan, 1977) and drugs for treating babesiosis are not readily available (Mount et al., 1991).

New approaches for tick population control are necessary due to the limited number of effective acaricides labeled for use in the United States, the constant threat of reinfestation, and the growing resistance of *Boophilus* ticks to currently available acaricides. One proposed method is the utilization of sterile hybrid males in a biological control system (Thompson et al., 1981). Graham and Price (1966) first described naturally occurring hybrids between *B. microplus* and *B. annulatus* based on morphological variation in ticks collected in northern Mexico. Laboratory crossmating studies revealed that *B. microplus* and *B. annulatus* hybridize readily without loss of fecundity in the P_1 generation (Graham et al., 1972). Resulting F_1

males were sterile because of malformed testes resulting in non-functional spermatozoa. First generation females show a decrease in fertility of approximately 50% (Newton et al., 1972a) which Newton et al. (1972b) report is likely due to meiotic malsegregation and chromosomal aberrations. First generation males and females have been found to mate readily with either parental type or other F_1 ticks (Newton et al., 1972a). Offspring of F_1 females show a reduced fertility in females and sterile males (Thompson et at., 1981). Back crossing showed F_3 generation females to be fully fertile. Six generations of backcrossing were required to return fertility of males to original levels (Thompson et al., 1981).

Boophilus microplus (Oliver and Bremner, 1968) and *B. annulatus* (Newton et al., 1972) have diploid numbers of 21 in males and 22 in females with an XX:XO sex determination system. Newton et al. (1972a) showed these two species have very similar karyotypes with autosomal length averages ranging between 2.3 and 4.9μ in *B. microplus* compared to 2.9 and 4.5μ in *B. annulatus*. A large difference was noted in length of the sex chromosome with that of *B. microplus* being shorter than that of *B. annulatus* (5.6 and 7.1μ respectively). The average length of the *B. annulatus* X chromosome compared to the average length of the longest and the shortest autosomes was 1.6:1 and 2.5:1 respectively. The ratios of the *Boophilus microplus* average length comparisons were 1.2:1 (X:longest autosome) and 2.4:1 (X: shortest autosome).

Similarity in standard karyotypes makes it necessary to differentially stain these karyotypes to examine chromosomal homology between the two species (Gunn

and Hilburn, 1989). Two such differential staining techniques are C- and G-banding. Using C- and G-banding techniques Gunn et al. (1993) determined the small *B. microplus* X chromosome to be, with the exception of one band, homologous to a portion of the large X chromosome of *B. annulatus*. Gunn et al. (1993) hypothesize the portion of the *B. annulatus* X chromosome not present in *B. microplus* X chromosome should occur within euchromatin of the autosomes of *B. microplus*. This would be supported if C- or G-banding patterns show homology between the X chromosome of *B. annulatus* and an autosome (or autosomes) of *B. microplus*.

Hilburn et al. (1989) used a silver staining technique to stain the nucleolar organizer regions (NOR) of *B. annulatus*, *B. microplus* and the hybrids. They found both species to have two active NORs while hybrids have only one. They also included C-band data which indicate all chromosomes to be acrocentric with *B. annulatus* having only centromeric heterochromatin and *B. microplus* having additional interstitial heterochromatin on three pairs of autosomes.

Observations of diakinesis in hybrid cells made by Newton et al. (1972b) exhibited four aberrations: 1) improper pairing of the X chromosomes, 2) a heteromorphic autosomal bivalent, 3) a possible trivalent, 4) dissociation of a bivalent lacking chiasmatic formation. Some possible explanations for these aberrations include translocation, duplication, heterochromatin additions and rearrangement between the karyotypes of the two species.

One goal of this research was to provide a base of knowledge about the cytogenetics of *B. microplus* and *B. annulatus* which included establishing standard

chromosome numbering for each species. Understanding the basic genetics of an organism is important if genetic make-up is to be used in its biological control. Information from C- and G-bands could help in assignment of genes and mutations to chromosome positions, these genes and mutations could in turn be used in population control and eradication programs. An example of genetically based population control is the release of sterile hybrids into natural populations. *Cochliomyia hominivorax* (screwworms) have been completely eradicated from the United States using sterile release technology. The G-band technique developed in this study could also be used to gain further knowledge about other veterinary and medically important species of ticks.

The specific goal of this project was to utilize G-bands of *B. microplus* and *B. annulatus* to locate the portion of the X chromosome of *B. annulatus* not included in the *B. microplus* X chromosome, explain meiotic aberrancies observed by Newton et al. (1972b) and determine the level of homology between the two species. This information would provide a basis for sterility observed in hybrids of the two species which is currently unknown. Hybrids are being studied for possible use in biological control.

MATERIALS AND METHODS

Specimens of *Boophilus microplus* and *B. annulatus* were obtained from Dr. Ron Davey at the United States Department of Agriculture-Agricultural Research Service, Cattle Fever Tick Research Laboratory, Moore Air Base Mission, Texas, through a Memorandum of Understanding (NO. 58-6206-1-1). This agreement provided for supplying of ticks at appropriate developmental stages for karyotypic investigation and use of their facilities as needed for dissection purposes.

The karyotypic technique utilized was preformed as per Gunn and Hilburn (1989). Fed nymphal ticks were dissected in modified Ringers solution (Morgan and LaBrecque, 1964) using a dissecting microscope. Gonadal tissues and sex glands were removed, placed in a hypotonic solution (0.075 M potassium chloride) on a vinyl slide, and allowed to incubate at room temperature for 20 minutes. These tissues were then transferred to a glass slide under the dissection microscope and any extra fluid was wicked off with a commercial tissue. A single drop of dissociation fluid (1 part lactic acid: 1 part acetic acid: 1 part distilled water) was added and gonadal tissues were chopped using minuten pins. After approximately 10 to 30 seconds cells were observed streaming away from the tissue clump and the dissociation was considered complete. Dissociated tissues were then squashed using a siliconized cover slip by applying firm pressure with the thumb for approximately 30

seconds. The coverslip was removed by flicking it off with a razor blade. All slides used in C-banding procedures and initial attempts of G-banding procedures were placed in Carnoy's fixative (6 parts methanol: 1 part acetic acid) for 15 minutes, removed and allowed to air dry. Later slides used in G-banding procedures were fixed in 3 parts acetic acid: 1 part methanol for five minutes (Steiniger and Mukherjee, 1975).

Before staining, all slides to be C-banded and initial slides to be G-banded were previewed on an Olympus Vanox-S research microscope. Phase contrast microscopy was used to determine the number of chromosomes present, the level of contraction, and the number of usable chromosome spreads. Slides with fewer spreads or incomplete spreads were used to establish approximate times for treatment in banding procedures. Later slides used in G-banding procedures were not previewed because the technique required application of banding procedures within 24 hours which did not allow enough time for slide previewing.

C-bands were produced for several individuals of both species of ticks using the C-banding procedure of Sumner (1972) as modified by Gunn and Hilburn (1989). Gunn and Hilburn's (1989) protocol required aging of slides for 14-60 days prior to applying C-banding procedures. Slides were placed in 0.2 N hydrochloric acid for 10 minutes, rinsed in two changes of distilled water and allowed to air dry. Next slides were immersed in 5% barium hydroxide for 1-10 minutes at temperatures varying from room temperature to 37° C depending on firmness of fixation. Softer fixation required lower temperatures and shorter times. The slides were then washed again in

0.2 N hydrochloric acid, rinsed in two changes of distilled water and allowed to air dry. Slides were then placed in 2X standard saline citrate (150mM NaCl, 15mM NaCitrate) 60° C for one hour, rinsed in two changes of distilled water and allowed to air dry. Finally, slides were stained in 4% Giemsa (R-66) in phosphate buffer for five minutes.

The G-banding technique used was as per Verma and Babu (1989) for G-bands by trypsin using giemsa (GTG). All slides were aged overnight with initial slides aged at 55° to 60° C and later slides aged at room temperature. Slides were subsequently treated in a 0.05% trypsin solution made with Dulbecco's phosphate-buffered saline-calcium and magnesium free (PBS-CMF) (Dulbecco and Vogt, 1957) for 1 to 10 seconds. Slides were then rinsed in Dulbecco's PBS-CMF kept in the refrigerator at 2° to 5° C. Slides were finally stained in 5% Giemsa in Gurr's pH 6.8 buffer (Sharma and Sharma, 1965) for 4 to 6 minutes, rinsed in distilled water and allowed to air dry.

Black and white photographs were taken and enlarged to a consistent size using a 3:5 (*B. microplus: B. annulatus*) ratio for the X chromosomes as established by Gunn et al. (1993). Chromosomes of C-banded karyotypes were cut out and matched based on length and positive heterochromatin regions. C-banded karyotypes of *B. microplus* were compared to C-banded karyotypes of *B. annulatus* and any differences in location of positive heterochromatin were noted. Chromosomes of G-banded karyotypes were cut out and matched based primarily on linear banding patterns. C-banded karyotypes were then correlated with the G-banded karyotypes of

the same species. G-banded karyotypes of the two species were then compared taking heterochromatin positive areas into account.

Previously published (Hilburn et al. 1989) silver stained karyotypes of both species were cut and correlated based on length and nucleolar organizer region (NOR) position. Silver stained karyotypes were then correlated with G-banded karyotypes to determine location of the NOR.

All chromosomes of both species were measured using Vernier calipers and averages were computed. These measurements were then used to number chromosomes according to length with the longest autosome being numbered one and the shortest being numbered ten. Ratios were then established between the lengths of the X chromosomes and the longest and shortest autosomes for both species.

RESULTS

Specimens of *Boophilus annulatus* (N=102 ♂, N=88 ♀) used in this study had a diploid number of 21 for males and 22 for females. Karyotypes were comprised of 20 autosomes with an XX:XO sex determination system (Figure 1). Measurements of the sex chromosome, longest autosome, and shortest autosome of 12 individuals were taken and average lengths were calculated. The sex chromosome was longer than the longest and shortest autosomes by ratios of 3.2:1 and 6.6:1 respectively. Seven individuals were C-banded with no intraspecific variation in placement of heterochromatin noted. Based on C-bands all chromosomes were acrocentric with only centromeric heterochromatin observed (Figure 1b).

Specimens of *B. microplus* (N=223 ♂, N=137 ♀) used in this study had a diploid number of 21 for males and 22 for females. Karyotypes were comprised of 20 autosomes with an XX:XO sex determination system (Figure 2). Measurements of the sex chromosome, longest autosome, and shortest autosome of twelve individuals were taken and average lengths were calculated. The sex chromosome was longer than the longest and shortest autosome by ratios of 1.7:1 and 2.9:1 respectively. Eleven individuals were C-banded with no intraspecific variation noted. All chromosomes were acrocentric with a single interstitial band of noncentromeric heterochromatin located proximal to the centromere approximately 1/4 of the way down the chromosome arm on chromosome pairs 6, 8 and 9 (Figure 2b).

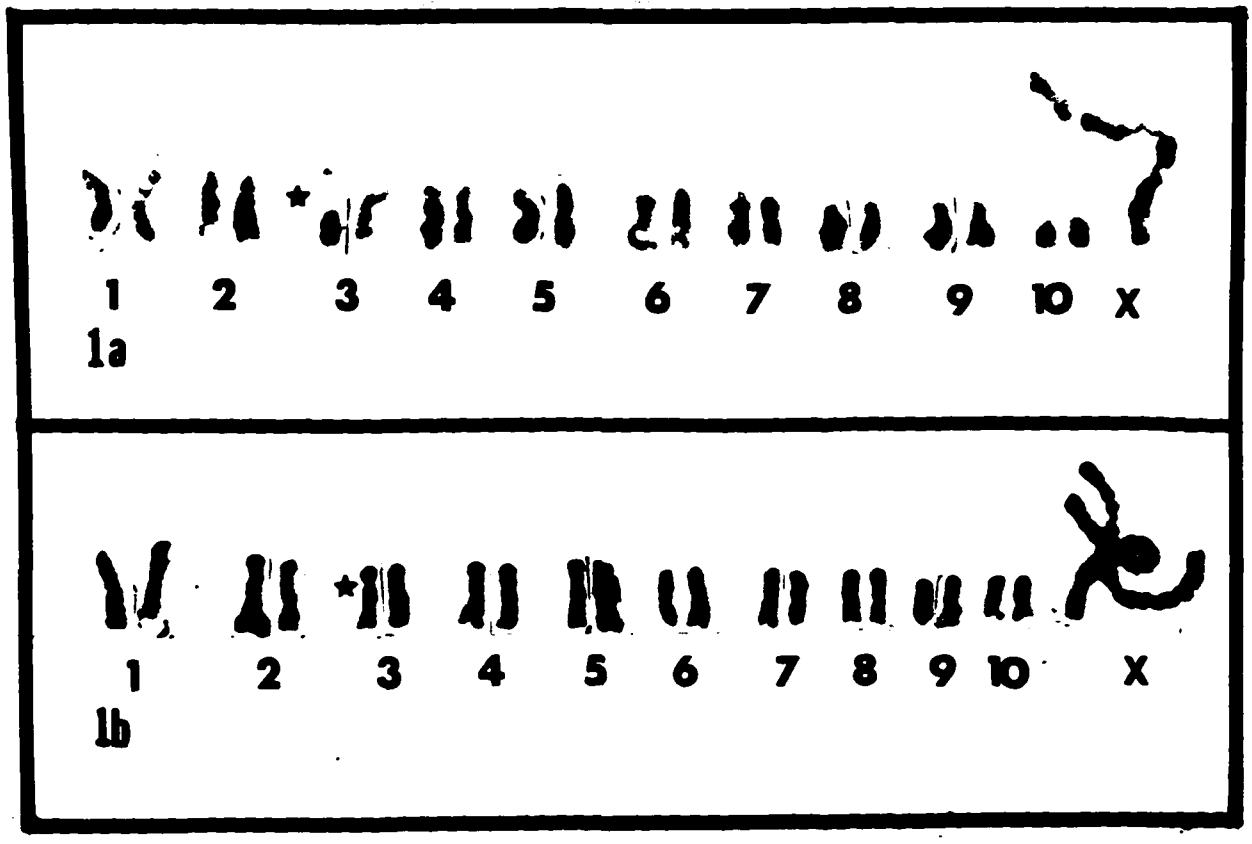

Fig. 1. a) G-banded and b) C-banded karyotypes of *Boophilus annulatus*. ★indicates NOR location.

Fig. 2. a) G-banded and b) C-banded karyotypes of *Boophilus microplus*. ★indicates NOR location. < indicates location of interstitial heterochromatin.

Several individuals of *B. annulatus* (N=6) and *B. microplus* (N=6) were successfully G-banded. G-banded karyotypes fixed in 3:1 acetic acid-methanol produced clearer banding patterns more consistently than those fixed in 6:1 methanol-acetic acid. Fixation solution had no obvious affect on G-band patterns. Figures 1a and 2a show representative G-banded karyotypes from single cells of *B. annulatus* and *B. microplus* respectively. Figures 3 and 4 are composite partial karyotypes comparing G-banded chromosomes of *B. annulatus* and *B. microplus*. In the composite figures each matched pair has a *B. annulatus* chromosome to the left and a *B. microplus* chromosome to the right.

Seven pairs of autosomes are G-band (Figure 3) and C-band (Figure 1b and Figure 2b) homologous between species. Length was used to determine chromosome numbering of both species. Chromosomes in figures 3 and 4 are paired based on G-band homology without regard to chromosome numbers. The *B. microplus* karyotype has two number shifts relative to *B. annulatus* due to additions of interstitial heterochromatin at chromosomes 6 and 9. A heterochromatin addition affected the length of *B. microplus* chromosome 6 which was longer than chromosome 7 although chromosome 7 was G-band homologous to *B. annulatus* chromosome 6. The same situation was seen in *B. microplus* chromosome 9 which was longer than chromosome 10 although chromosome 10 was homologous with *B. annulatus* chromosome 9. Three pairs of autosomes differ only in the addition of noncentromeric heterochromatin in *B. microplus* (Figure 4).

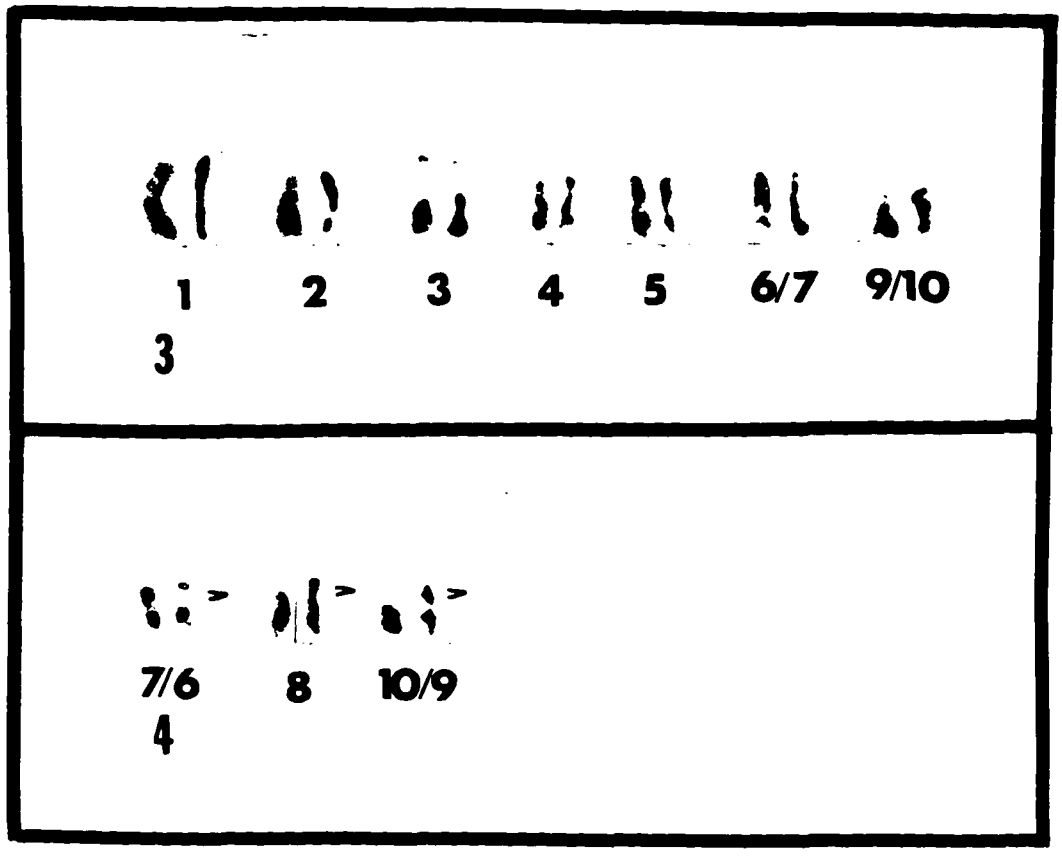

Fig. 3. Composite partial karyotype comparing G-banded chromosomes of *Boophilus annulatus* and *B. microplus*, showing G-band homologous chromosomes. Differences in numbering was due to length added by interstitial heterochromatin added to *B. microplus* chromosomes 6 and 9 which was not found in the *B. annulatus* karyotype. In each pair the chromosome on the left was from *B. annulatus* and the chromosome to the right was from *B. microplus*.

Fig. 4 Composite partial karyotype comparing G-banded chromosomes of *B. annulatus* and *B. microplus*, showing those chromosomes which differ in the presence (*B. microplus*) of interstitial heterochromatin. Differences in numbering were due to the length added by this heterochromatin. In each pair the chromosome on the left was from *B. annulatus* and the chromosome to the right was from *B. microplus*. > indicates location of interstitial heterochromatin.

Chromosome 3 in both species showed variation in length at an area of extreme constriction, the range of variation is displayed in Figure 5. The nucleolar organizer region (NOR) was located on chromosome 3 in both species and is indicated in Figures 1 and 2.

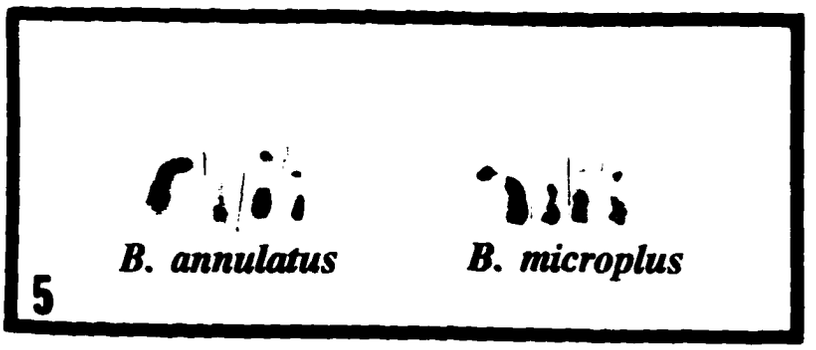

Fig. 5. Variation in the NOR bearing chromosome 3 in *Boophilus annulatus* and *B. microplus*.

DISCUSSION

A karyotype consisting of 20 autosomes with an XX:XO sex determination system resulting in diploid numbers of 21 for males and 22 for females supports the findings of Oliver and Bremner (1968) for *Boophilus microplus*. These chromosome numbers are consistent with the findings of Newton et al (1972a), Gunn and Hilburn (1989), Hilburn et al. (1989), and Gunn et al. (1993) for both species.

The *B. annulatus* sex chromosome was proportionally much longer than reported by Newton et al. (1972a) with the ratios of the sex chromosome to the longest autosome reported to be 1.6:1 versus 3.2:1 observed in this study and the ratio with the shortest autosome reported to be 2.5:1 versus 6.6:1 observed in this study. The use of karyotypes undergoing different cellular processes may account for the differences in X chromosome length ratios. Newton et al. (1972a) based their measurements on an unspecified number of meiotic metaphase I cells while this study utilized mitotic metaphase chromosomes. There was also a difference in the proportionate size of the *B. microplus* X chromosome with Newton et al. (1972a) reporting the ratio between the longest autosome and the sex chromosome to be 1.2:1 versus 1.7:1 observed in this study and between the shortest autosome and the sex chromosome to be 2.4:1 versus 2.9:1 observed in this study.

Hilburn et al. (1989) reported C-band data for both species and indicated all chromosomes were acrocentric. Heterochromatin was restricted to the centromeric

regions in *B. annulatus* while *B. microplus* had centromeric heterochromatin plus a single additional band of interstitial heterochromatin on chromosome numbers 2, 4 and 10 with numbering based on size. The C-band data of this study agree with those of Hilburn et al. (1989) in number and location of bands with the only difference being chromosome numbering. Differences in numbering result from difficulty of numbering chromosomes that are very similar in size. This study utilized many more karyotypes and G-band data which provided more accurate measurement and numbering of individual chromosomes. The three *B. microplus* chromosomes with interstitial heterochromatin were numbered 6, 8 and 9 to correspond to G-band pattern. Positive C-bands denote areas of constitutive heterochromatin which consists of highly repetitive DNA that may be rich in adenine and thiamine (AT), cytosine and guanine (CG) or neutral (AT-CG mixed) (Comings, 1978). G-band positive regions indicate areas of intercalary heterochromatin that are generally AT rich. All interstitial C-band positive regions were located in G-band negative regions (Figure 2) indicating the interstitial C-bands of these species are either CG rich or neutral.

Comparison of silver stained chromosomes with G-banded karyotypes indicate the NOR of both species to be on chromosome number 3 which is G-band homologous between species. Chromosome number 3 is the autosome with constriction just below the centromere reported by Hilburn et al. (1989). The location of the NOR is the area of varied constriction displayed in Figure 5. Interspecific homology of G-bands of this chromosome indicates that the single NOR staining in the hybrids is possibly due to differential expression or activation (Hilburn et al.,

1989). The NOR is important because it is the site of ribosomal RNA production which is essential in protein production.

C- and G-band results combined with the previously published G-bands of the X chromosomes reported by Gunn et al. (1993) appear to indicate high interspecific homology with the only differences between the two species being accounted for by addition of heterochromatin to *B. microplus* chromosomes 6, 8 and 9. Homologous pairing should occur in hybrids during meiosis as follows: The smaller X chromosome of *B. microplus* should pair readily with the G-band homologous region of the larger *B. annulatus* X chromosome as suggested by Gunn et al. (1993). The single G-band observed in the X chromosome of the *B. microplus* not found in the *B. annulatus* X may cause an asynaptic bulge to occur during meiotic pairing. Chromosomes 1 through 5, *B. annulatus* 6 and *B. microplus* 7, and *B. annulatus* 9 and *B. microplus* 10 should pair during meiosis with complete homology (Figure 3). The additions of interstitial heterochromatin in *B. microplus* 6, 8 and 9 may cause asynaptic bulges to appear during meiotic pairing with *B. annulatus* chromosomes 7, 8 and 10 respectively (Figure 4).

The G-band results, combined with the high degree of G-band homology of the X chromosomes reported by Gunn et al. (1993), suggest that meiotic aberrancies observed in hybrids by Newton et al. (1972b) are not due to lack of chromosomal homology. There are several other possible explanations for the observations of Newton et al. (1972b). Aberrancies may be due to the squash technique used in preparing tissue. Squash techniques involve flattening of three dimensional structures

of the cell into two dimensions and could cause distortion of structures. Another possible explanation for these aberrancies is that they may be rare occurrences. Newton et al. (1972b) based their findings on aberrancies in 5 specimens (of 58) each having from 1 to 4 of the aberrancies discussed. A third possible explanation for the lack of support for the meiotic aberrancies may be differences in the specimens themselves. Newton et al. (1972b) examined hybrids of known parentage from a field laboratory in Nuevo Laredo, Tamaulipas, Mexico. This study utilized strains maintained from outbreaks in southern Texas. Newton et al. (1972b) reported the testis of F_1 males to either be absent or malformed. The testis of F_1 males produced by crossing strains used in this study were reduced in size but were otherwise normal (Gunn, S. J., pers. comm.).

Results of this study did not locate euchromatin from the area of the *B. annulatus* X chromosome, which is not included in the *B. microplus* X chromosome, within the *B. microplus* autosomes. There were no areas of interspecific autosomal difference that could not be accounted for by additional interstitial heterochromatin found in the genome of *B. microplus*. Chromatin missing in the *B. microplus* genome may be lost indicating a greater efficiency in the remainder of the *B. microplus* genome. Another possible explanation is that the chromatin found on the larger *B. annulatus* X chromosome is repeated at other locations in its genome. Karyotypes of both species were examined for this possibility but there were no areas of homology between the X chromosome of *B. annulatus* found within the autosomes of either species. It would be possible to locate the remaining chromatin by developing DNA

probes for areas of the *B. annulatus* X chromosome not found in the *B. microplus* X chromosome. Once produced these probes could be fluorescently labeled and hybridized into the genomes of both species. The hybridized probes would identify the location of the missing euchromatin from the *B. annulatus* X chromosome in the *B. microplus* genome. Chromosome numbering from this study will enable researchers to accurately describe locations of these areas. Establishment of either the location of the euchromatin found on the *B. annulatus* X chromosome that is not found on the *B. microplus* X chromosome or its absence in the *B. microplus* genome is important if the function of this DNA is to be understood.

There have been no published studies on the release and mating success of sterile *Boophilus* hybrids in a natural setting, all mating studies have been performed in laboratory settings. Studies using releases of sterile hybrids into isolated areas are essential prior to implementation of hybrid technology in biological control. To date, there have been studies to determine mating competitiveness of hybrids versus pure-strains of both species (Davey, 1986). Type II hybrid (*B. annulatus* ♂ X *B. microplus* ♀) males and pure-strain males were equally successful in mating while type I hybrid (*B. microplus* ♂ X *B. annulatus* ♀) males could not successfully compete with either pure strain. This difference in reproductive ability indicates that hybrids to be released into natural populations as a biological control should be type II. Isolation of the study area is essential in preventing reinfestation of pure strains. Assessing existing infestation level is also important because if sterile hybrids are to function in biological control they must out number fertile wild ticks by a 2:1 ratio

(Osburn and Knipling, 1982). Sterile hybrids could not be used as a method of control in areas with high numbers of wild ticks due to the amount of tick burden on cattle.

If proven effective, sterile release could eventually be used in conjunction with other control methods enabling the eradication of *Boophilus* ticks in situations where not all live stock can be gathered and dipped and where native wildlife act as hosts. Integrated systems of control are more plausible than any single method of control. Pesticides would be used to lower infestation levels to a point where introduction of enough sterile hybrids would be possible. Sterile hybrid technology would then be used to control populations of wild ticks on wildlife and livestock that could not be dipped.

Other livestock pests have been successfully eradicated using release of sterile individuals into wild populations. For example, *Cochliomyia hominivorax* (screwworm) was eradicated using releases of sterile screwworm flies produced by irradiation of the larval stage (Graham and Hourrigan, 1977). Screwworms were quickly eliminated in the southeastern United States by releasing sterile flies because distribution was limited to southern Florida which was easily isolated. Screwworms were also readily eradicated in Puerto Rico and the American and British Virgin Islands. Eradication in the Southwest was more difficult due to constant reinfestation by flies from northern Mexico. In 1972, the Mexico-American Screwworm Eradication Program began and enough sterile flies were produced in southern Mexico and Mission, Texas to eradicate screwworm populations in southwestern United States

and northern Mexico. Screwworms are damaging to livestock as larvae but are harmless as adults. Sterile screwworms released were adult flies which add no burden on livestock so overwhelming wild populations with sterile flies was possible.

Biological control of many species of ticks with veterinary and medical importance will be furthered by the G-band technique developed in this study. G-banding can now be used to provide accurate numbering and descriptions of genomes of ticks. Numbering and genomic description are used as a basis for chromosome mapping and molecular investigations requiring identification of specific areas or segments of DNA within the genome. Information provided by chromosome banding can also function as an independent test of current phylogenetic classification of related species which would aid in describing genomic evolution of various groups.

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