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GENETIC INCOMPATIBILITY BETWEEN *BOOPHILUS DECOLORATUS* (KOCH, 1844) AND *BOOPHILUS MICROPLUS* (CANESTRINI, 1888) AND HYBRID STERILITY OF AUSTRALIAN AND SOUTH AFRICAN *BOOPHILUS MICROPLUS* (ACARINA: IXODIDAE)

A. M. SPICKETT[1] and J. R. MALAN[2]

ABSTRACT

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Virgin females of both *Boophilus decoloratus* and *Boophilus microplus*, when mated with males of the other species, subsequently produced sterile eggs. Counts of spermiophore capsules in female seminal receptacles showed that the males of both species will mate with the females of both species and that *B. microplus* males show a slightly greater, but statistically insignificant, mating capacity than *B. decoloratus* males. South African *B. microplus* females, when mated with an Australian strain of *B. microplus* males, produced a 62% yield of viable hybrid progeny while the reciprocal cross produced only a 1,82% hatch of non-viable larvae. The hybrids were sterile when interbred and no hatch resulted when the Fl males were backcrossed with parent females. The reciprocal backcross of hybrid Fl females to parent males resulted in a low percentage hatch of non-viable larvae.

Résumé

INCOMPATIBILITÉ GÉNÉTIQUE DE BOOPHILUS DECOLORATUS *(KOCH,* 1844) *ET* BOOPHILUS MICROPLUS *(CANESTRINI,* 1888). *STÉRILITÉ DES HYBRIDES DE SOUCHES AUSTRALIENNES ET SUD-AFRICAINES DE* BOOPHILUS MICROPLUS *(ACARINA: IXODIDAE)*

Des femelles vierges, tant de Boophilus decoloratus *que de* Boophilus microplus, *n'ont produit que des oeufs stériles après avoir été accouplées à des mâles de l'autre espèce. On a constaté en dénombrant les capsules spermiophoriques dans les réceptacles séminaux de la femelle que les mâles des deux espèces prennent indifféremment les femelles de l'une et de l'autre; ceux de* B. microplus *ensemencent un peu plus de femelles que ceux de* B. decoloratus, *mais la différence n'est pas significative. Des femelles d'une souche sud-africaine de* B. microplus *accouplées à des mâles d'une souche australienne de la même espèce ont eu une progéniture viable à 62% tandis que le croisement réciproque n'a abouti qu'à un pourcentage d'éclosion de* 1,82, *les larves n'étant du reste pas viables. Les hybrides sont inter-stériles: aucune éclosion n'a résulté du rétrocroisement de mâles F*1 *avec des femelles parentes; quant aux femelles F*1 *accouplées à des mâles parents, leur ponte accuse un faible pourcentage d'éclosion, mais les larves ne sont pas viables.*

INTRODUCTION

Boophilus decoloratus (Koch, 1844) and *Boophilus microplus* (Canestrini, 1888), both of which are 1-host species with a preference for cattle as hosts, are economically important in South Africa mainly because they transmit the pathogenic bovine parasites causing redwater (*Babesia* spp.), gallsickness (*Anaplasma marginale*) and spirochaetosis (*Borrelia theileri*) (Hoogstraal, 1956; Theiler, 1962). They co-exist in some parts of the country, *B. decoloratus* being by far the more widely-distributed of the 2 species. *B. microplus*, however, appears to be spreading and is now well established in some areas from which only *B. decoloratus* was recorded previously (Theiler, 1949, 1962; Howell, Walker & Nevill, in press). Because of the close relationship between these 2 species it was decided to investigate the possibility of hybridization between them.

Preliminary work on acaricide resistance in which a strain of *B. microplus* from Australia had been cross-mated with a strain from South Africa suggested that a degree of incompatibility exists between these strains. The cross-mating experiments described in this report were initiated in an attempt to determine this degree of incompatibility.

MATERIALS AND METHODS

Boophilus ticks used in these experiments were *B. decoloratus* "High Hill" strain, routinely bred in this laboratory; South African *B. microplus* "Wonderboom" strain (referred to hereafter as *B. microplus* S.A.), collected north of Pretoria (28°15'E, 25°39'S) and maintained in the laboratory; and Australian *B. microplus* "Yeerongpilly" strain, kindly supplied by CSIRO, Division of Entomology, Indooroopilly, Australia (*B. microplus* A).

Virgin males and females were obtained by feeding larvae of the different strains on separate calves, removing the pharate nymphs and allowing them to moult individually in small vials in an incubator (26 °C; 90% R.H.). Freshly emerged adult ticks were fed in containers applied to the backs of calves with a contact adhesive.

In the 1st series of experiments designed to determine whether the males do in fact inseminate females of the other species or strain, cardboard containers 9 cm in diameter were used. With the *B. decoloratus* and *B. microplus* S.A. cross 1 male and 10 females (5 *B. decoloratus* and 5 *B. microplus*) were placed in each container. Males were thus provided with a choice of females to determine whether they will mate with the other species if they are not in a forced mating situation. The experiment was replicated 5 times with each species of male.

With the intraspecific South African and Australian *B. microplus* cross, each male was constantly provided with 8 females of the other strain, a total of 10 replicates being carried out. The resulting engorged, detached females were dissected and counts made of male spermiophore capsules present in their seminal receptacles to determine whether insemination had taken place.

The 2nd series of experiments was carried out in plastic containers 5 cm in diameter applied to the backs of calves. Freshly emerged adults were placed in the containers in the desired sex and species combinations. Initially 1 male and 4 females were

(1) Department of Entomology, Veterinary Research Institute, Onderstepoort, 0110, South Africa

(2) P.O. Box 502, Cullinan, 1000, Transvaal, South Africa

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placed in each container. Those that died or failed to attach were replaced until no more freshly emerged ticks remained. The following 7 combinations were made:

Females × Males

B. decoloratus × 1. *B. decoloratus*
2. *B. microplus* S.A.

B. microplus S.A. × 1. *B. decoloratus*
2. *B. microplus* S.A.
3. *B. microplus* A.

B. microplus A. × 1. *B. microplus A.*
2. *B. microplus* S.A.

Backcross matings were later done in the same manner and F1 matings were done free on an animal. The resulting engorged detached females were placed individually in glass tubes and allowed to oviposit in an incubator (26 °C; 90% R.H.). The number of eggs produced by each female and the percentage hatch of each egg batch were determined 3 months later to avoid disturbing the females during oviposition.

RESULTS

First series of experiments (insemination by males)

A. *B. decoloratus* × *B. microplus* S.A.

Tables 1 and 2 respectively show the results of matings of single *B. decoloratus* and *B. microplus* S.A. males with females of both the species. Males of both species are capable of inseminating both *B. decoloratus* and *B. microplus* females, mating indiscriminately with any available female. *B. decoloratus* males showed an 8,4% preference for females of their own species while *B. microplus* males inseminated 3,4% more *B. decoloratus* than *B. microplus* females. These preferences, however, were not statistically significant [*B. decoloratus*, t=1,26 and *B. microplus*, t=0,41: t (0,05), v=8,=2,306] (Tables 1 and 2).

B. microplus males inseminated more females (mean 5,8±0,58) than *B. decoloratus* males (mean 4,8±0,37), but this difference was not statistically significant [t=—1,44:t (0,05), v=8,=2,306].

All females inseminated by *B. decoloratus* males (Table 1) yielded either 1 or 2 male spermiophore capsules, 1 capsule being found in significantly more females (70,8%) than 2 capsules [t=2,36:t (0,05), v=8,=2,305].

Females mated with *B. microplus* males (Table 2) also yielded either 1 or 2 male spermiophore capsules but the occurrence of 1 capsule (62,1% of females) compared to that of 2 is not statistically significant [t=1,07:t (0,05), v=8,=2,306].

B. *B. microplus* S.A. × *B. microplus* A.

Table 3 clearly shows that South African and Australian *B. microplus* males are capable of inseminating females of the other strain, the South African males inseminating 70% and the Australian males 72,5% of the females available to them.

Second series of experiments (cross-matings)

A. *Normal, control matings*

The mean number of eggs produced by engorged, detached females obtained from each of the 3 normal control matings and the mean percentage hatch are shown in Table 4. No significant difference exists in the oviposition capacity of normally mated *B. decoloratus* and *B. microplus* S.A. females [t=—1,53: t (0,05), v=38, =2,021], or in that of *B. microplus* S.A. and *B. microplus* A. females [t=0,19: t (0,05), v=38, =2,021].

B. *B. decoloratus* × *B. microplus* S.A.

The results of the 2 interspecific cross-matings between *B. decoloratus* and *B. microplus* S.A. are shown in Table 5. No larval hatch was recorded from any of the egg batches produced by females mated with males of the other species although some incomplete larval development took place within the eggs. South African *B. microplus* and *B. decoloratus* are thus genetically totally incompatible, although the males of both species are not species-specific as regards mating and insemination of females of these 2 species (Tables 1 & 2).

TABLE 1 Results of matings of 5 *B. decoloratus* males each supplied with 5 *B. decoloratus* and 5 *B. microplus* S.A. females

Females	South African		Total	"t" values
	B. decoloratus females	*B. microplus* females		
Total number used	5×5	5×5	5×10	
No. fertilized	13	11	24	
Mean No. fertilized±SE	2,6(±0,24)	2,2(±0,20)	4,8(±0,37)	1,26
Mean No. with 1 sperm capsule±SE	1,6	1,67	3,4(±0,75)	
Mean No. with 2 sperm capsules±SE	1,0	0,4	1,4(±0,40)	2,36

TABLE 2 Results of matings of 5 *B. microplus* S.A. males each supplied with 5 *B. microplus* S.A. and 5 *B. decoloratus* females

Females	South African		Total	"t" values
	B. decoloratus females	*B. microplus* females		
Total number used	5×5	5×5	5×10	
No. fertilized	15	14	29	
Mean No. fertilized±SE	3(±0,32)	2,8(±0,37)	5,8(±0,58)	0,41
Mean No. with 1 sperm capsule±SE	2,0	1,6	3,6(±1,08)	
Mean No. with 2 sperm capsules±SE	1,0	1,2	2,2(±0,73)	1,07

Oviposition was effected in that cross-mated females of both species laid fewer eggs than normally-mated control females, though this difference was not statistically significant [*B. decoloratus*, t=0,65 and *B. microplus*, t=0,45: t (0,05), v=38, =2,021].

TABLE 3 Results of matings of South African (S.A.) and Australian (A) *B. microplus* males with Australian and South African *B. microplus* females respectively. Each male was supplied with 8 females

	Mating performed	
	A. ♀×S.A. ♂	S.A. ♀×A. ♂
No. males used	10×1	10×1
No. females used	10×8	10×8
No. females fertilized	56	58
Mean No. females fertilized	5,6	5,8
Mean No. females with 1 sperm capsule	3,2	3,6
Mean No. females with 2 sperm capsules	2,4	2,1
Mean No. females with 3 sperm capsules	0	0,1

TABLE 4 Results of the 3 normal control matings performed

	Mating performed		
	South African *B. dec.* ♀ × *B. dec.* ♂	South African *B. mic.* ♀ × *B. mic.* ♂	Australian *B. mic.* ♀ × *B. mic.* ♂
No. of females	20	20	20
Mean No. eggs± SE	2 974 (±98)	3 160 (±73)	3 140 (±76)
Mean % hatch	90,2	87,7	91,9

TABLE 5 Total egg production per female and percentage hatch per egg batch resulting from the 2 interspecific cross-matings performed

♀ No.	Crossing performed			
	South African *B. dec.* ♀ × *B. mic.* ♂		South African *B. mic.* ♀ × *B. dec.* ♂	
	Eggs	% Hatch	Eggs	% Hatch
1	3 140	*	2 794	*
2	2 790	*	3 421	*
3	1 856	*	3 176	*
4	2 476	*	2 394	*
5	3 160	*	2 975	*
6	2 846	*	3 004	*
7	2 765	*	2 643	*
8	3 048	*	3 219	*
9	2 672	*	3 378	*
10	3 240	*	3 142	*
11	3 254	*	2 854	*
12	3 336	*	3 096	*
13	2 301	*	3 214	*
14	2 975	*	2 571	*
15	3 130	*	3 400	*
16	3 270	*	3 094	*
17	2 945	*	2 673	*
18	2 571	*	2 482	*
19	2 934	*	3 324	*
20	3 104	*	2 914	*
Mean ± SE	2 890 (± 83)	0	2 988 (± 70)	0

* No hatch recorded

C. *B. microplus* S.A. × *B. microplus* A.

In the 2 intraspecific *B. microplus* cross-matings (Table 6), the mean hatch of eggs produced by *B. microplus* S.A. females mated with Australian males was 62,24% while the reciprocal cross yielded a mean hatch of only 1,82% larvae which died before they could be fed.

TABLE 6 Total egg production per female and percentage hatch per egg batch resulting from the 2 interspecific cross-matings performed (S.A.=South African; A.= Australian)

♀ No.	Crossing performed			
	S.A. *B.mic.* ♀ × A. *B. mic.* ♂		A. *B. mic* ♀ × S.A. *B. mic.* ♂	
	Eggs	% Hatch	Eggs	% Hatch
1	1 725	59,3	2 734	2,63
2	3 160	66,8	3 042	2,86
3	3 241	56,4	2 876	1,67
4	3 075	58,9	2 764	2,06
5	3 162	68,1	3 104	0,71
6	3 194	62,9	3 172	2,24
7	2 803	61,5	3 090	1,81
8	3 059	65,2	3 178	1,48
9	3 176	65,8	2 654	1,47
10	2 945	65,4	2 945	2,58
11	3 041	54,6	2 780	2,27
12	3 196	60,2	3 120	1,89
13	3 178	56,3	3 096	2,00
14	2 843	58,2	2 854	2,24
15	2 958	58,6	2 768	1,37
16	3 074	64,3	3 087	1,55
17	2 996	59,7	3 276	1,65
18	3 129	63,4	3 150	1,97
19	3 201	68,8	3 072	0,98
20	3 254	69,8	2 879	1,08
Mean ± SE	3 020 (± 74)	62,24	2 982 (± 40)	1,82

The Fl progeny (larvae) resulting from the South African female and Australian male mating were placed free on a bovine and the engorged females collected laid a mean of 90% of the number of eggs produced by normally mated *B. microplus* S.A. females, no hatch being recorded and no larval development being observed within the eggs. When the Fl females were backcrossed to South African parent males, an 11,2% larval hatch resulted from the eggs produced and, when backcrossed to Australian parent males, a 1,4% larval yield was recorded (Table 7). Larvae resulting from both backcross matings were not viable and died 4–8 days after hatching. The Fl males, when backcrossed to both Australian and South African parent females, were sterile as no larvae hatched (Table 7).

TABLE 7 Results of backcross of F1 (progeny of *B. microplus* S.A. females and *B. microplus* A. males) with South African (S.A.) and Australian (A.) parents

Crossing performed		% Eggs laid	% Hatch
♀	♂		
F1	S.A.	93,8	11,2
S.A.	F1	89,5	0
F1	A.	93,5	4
A.	F1	85,2	0

Discussion

Reports of interspecific mating of ixodid ticks are not uncommon in the literature. Pervomaisky (1954) reported that: *Rhipicephalus sanguineus* females and *Rhipicephalus bursa* males mate and produce progeny of which only females with criteria of the maternal form develop, the reciprocal cross proving sterile; *R. sanguineus* and *Rhipicephalus turanicus* interbreed and produce fertile progeny; *Hyalomma dromedarii* and *Hyalomma anatolicum* mate and produce sterile eggs; *Hyalomma plumbeum* males and *Hyalomma anatolicum* females interbreed and produce 16 hybrid generations, the reciprocal cross proving sterile, and *Hyalomma a. asiaticum* males and *H. a. caucasium* females interbreed and produce 3 hybrid generations. More recently Cwilich & Hadani (1963) reported that *Hyalomma excavatum* females and *H. marginatum* males mate and produce a fertile hybrid progeny while the reciprocal cross proved sterile. Oliver, Wilkinson & Kohls (1972) showed that *Dermacentor variabilis* females, mated to *Dermacentor andersoni* males, produce a progeny, the reciprocal cross being sterile, and that the cross-mating of *D. andersoni* females to *Dermacentor occidentalis* males is fertile, the reciprocal cross again being sterile. Kalyagin (1967) even reported a case of intergeneric copulation between male *Ixodes persulcatus* and female *Haemaphysalis concinna* but did not state whether insemination or spermatophore transfer took place. It is known, however, (Arthur, 1962; Balashov, 1956) that spermatogenesis is completed in the nymphal stage in the genus *Ixodes*, the males thus being able to mate upon emergence.

The cross-matings between *B. decoloratus* and *B. microplus* showed that these 2 species are totally incompatible, although the males of both species are not species-specific as regards mating and insemination. A certain degree of competition must exist where their distribution overlaps. *B. microplus* has only a slightly shorter life-cycle than *B. decoloratus* (Arthur & Londt, 1973; Spickett, unpublished data), the males displaying no significant difference in fertilization capacity and the females no significant difference in oviposition potential. The spread of *B. microplus* into areas where it has not been recorded previously and the seeming replacement of *B. decoloratus* in some coastal areas (J. A. F. Baker, 1977, personal communication) must therefore be due to other reasons such as adaptation to the environment, development of resistance to insecticides and favourable weather conditions.

The assumption by Londt & Spickett (1976) that *B. decoloratus* males are capable of producing either 1 or 2 spermiophore capsules per mating is supported in these experiments, but they also show that 1 capsule rather than 2 is produced more frequently per mating than was thought when spermiophore counts of females resulting from a single infestation of larvae on an animal were done. Londt (1976) surmised that a *B. decoloratus* male would probably inseminate a female once only if other virgin females were available. This is supported by the fact that no more than 2 spermiophore capsules were found in any of the inseminated females and probably also applies to *B. microplus* males, where the same situation exists.

Balashov (1971) recorded cases of decreased fertility and sterility in the progeny of *Ornithodoros tartakovskyi* after cross-mating the populations which were most widely separated geographically and which had practically no gene flow between them under natural conditions. Parthenogenesis has been reported for *B. microplus* by Stone (1963) but the much greater number of progeny obtained in these experiments are certainly hybrids. No gene flow exists between Australian and South African *B. microplus* and cross-mating shows a significant decrease in fertility to the extent that Fl interbreeding results in sterile eggs. The results of these intraspecific cross-matings conform more to the interspecific crossings mentioned above in that females of one strain (South African) mated to males of the other strain (Australian) yield some progeny while the reciprocal cross results in a very low percentage hatch. The reason for the failure of eggs of the reciprocal cross to hatch is unknown. Fertilized eggs should have the same genetic content irrespective of which strain provided the egg or the sperm, and the yield of progeny in only one direction (South African females × Australian males) suggests that the cytoplasm of the egg may play a role in the fertility of the cross-mating Backcross of the Fl hybrids obtained to the parent strains shows that the Fl males are completely sterile and that the parent males of both strains have diminished success when paired with Fl hybrid females.

According to Londt & Arthur (1975), only very slight morphological differences exist between Australian and South African *B. microplus* and they saw no reason to regard these 2 strains as separate species. The geographical isolation of these 2 strains, with the resulting absence of migratory gene flow and the existence of hybrid sterility shown here, would, however, suggest that they are sibling species which could be in the process of undergoing speciation.

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