

INDUCTION OF MALE STERILITY THROUGH MANIPULATION OF GENETIC MECHANISMS PRESENT IN VECTOR SPECIES OF TRIATOMINAE II: PARTIAL RESTORATION OF MALE FERTILITY.

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The development of integrated measures which involve sterile male release to supplement the conventional insecticidal techniques used in control against insects of medical importance, raised the question, whether the vectors of Chagas' disease possess the natural mechanisms by manipulation of which they may be controlled.

Results of earlier experiments, that had been published previously, were restricted to fragmentary information that raised various questions, the answer to which became available in the study herein described.

Interspecific hybrids were produced from reciprocal crosses between T. pseudomaculata and T. sordida and from unilateral crosses between female T. pseudomaculata and male T. infestans. These females mated with males, laid less than the normal complement of eggs, but offspring was relatively abundant. When T. pseudomaculata females were paired with T. brasiliensis males, hybridization was more difficult because few of the females mated and those that did had a strongly reduced fertility. Adults emerged from all crosses but exhibited sex disproportion, females predominating in all populations but one. The two Rhodnius species tested were also found to cross, but only when female R. prolixus were paired with male R. neglectus. These females laid a relatively high complement of eggs, had a strongly reduced fertility, but 50% of the fertile eggs developed into vigorous adults, males predominating females.

Neither type of hybrid male elicited fertilized eggs from either parental type of female, through their vesicula seminalis were found to be packed with spermatozoa, some normal looking and moving, others underdeveloped and motionless. Although, no artificial insemination was performed, the sperm in itself did not appear to be the prime inducer of sterility. Females paired with these hybrids did mate, sperm was transferred, as evidenced by the discharged spermatophores smeared with sperm, but did not contain spermatozoa in their spermatecae. The failure of the sperm to migrate to the spermatecae indicate prezygotic pos-copulation incompatibility, thus the hybrid male can't be used to suppress populations.

The female hybrids mated with parent males of either species had reduced fertility and their sons were sterile as were those of their fertile daughters. However, continous backcrossing of the hybrid females and their female progeny to parental males partially restored fertility of the males and increased fertility of females, as scored by egg hatchability. Fertility of hybrid females, measured by the yield of adults capable to reproduce, indicated that the reproductive performance decreased when hybrid females and their daughters were backcrossed additional generations to parental males. It is tentatively suggested that hybrid females could be used for suppression if they compete efficiently with wild females.

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** LABORATÓRIO DE BIOLOGIA e CONTROLE de VETORES da DOENÇA de CHAGAS. INERU, FUNDAÇÃO OSWALDO CRUZ, ESTR. DA COVANCA 56, JACAREPAGUÁ, R.J., BRAZIL. Presented at the Hybrid Sterility Symposium, XV International Congress of Entomology, Washington D.C. U.S.A. 1976.
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The attention which has been paid in the past to the various aspects of interspecific hybridization in Triatominae is mainly due to the importance of this phenomena in evolutionary theory^{1, 2, 8, 9, 14}. The purpose of experimental studies on the interspecific mating behaviour was to gather information and to discuss its implication in relation to the biological species concepts as postulated by Mayr et al⁷. Experimental crosses were made and once shown that hybrids were intermediate in morphological characters and were fertile as were their descendents, the polytypic species concept was applied^{13, 18}, thus reducing the number of full species and clearing the cases of allopatric populations as subspecies.

More recently the possibility of using genetic incompatibility to control insects of medical importance stimulated our interest in hybridization studies. The intriguing question being, whether Triatominae possess the natural genetic mechanisms by manipulation of which they can produce sterile males, release of which would substitute or complement the conventional insecticidal technique used in control of the vector of Chagas' disease. The information available on vector biology¹² and on vector behaviour, when analyzed in the light of the hypothetical model of integrated control measures stressed by Knipling^{5, 6}, suggest that this insect might be vulnerable to such an approach of control.

Laboratory and field reports indicate that either HCH or Dieldrin, when properly applied, can reduce the natural population to approximately 3% of pretreatment levels. Furthermore, these insects are short range migrants, thus treated areas are not subject so much to natural reinvasion as to accidental introduction of insects. After the population has been reduced to a low level, the release of sterile males, that would compete with survivors and those emerging from eggs, usually not affected by insecticides, appear feasible and more economical in preventing repopulation than the continuous sprayings year after year. But the painstaking work of raising successive generations of insects with a life cycle varying at least from 140-180 days in general, and in raising successive generations of hybrids backcrossed with parental males in particular, was the main reason for lack of information in this field of research. We

find no reference on this subject until 1972 when P. Szumlewicz published her results of preliminary experiments undertaken as a part of the program on Control of Chagas' disease in the Instituto Nacional de Endemias Rurais (INERu), Fundação Oswaldo Cruz.

Some of the material in this report is contained in the earlier paper¹⁰. Results of further experiments were presented at the Third International Congress of Parasitology in Munich Germany in 1974¹¹ and therefore it will be familiar to many. I have attempted to revise experiments still unfinished at the date of previous publication¹⁰, to add the material that appeared in the last four years and to integrate a few morphological, taxonomical and cytological observations that were not available before. The major contribution of the new material herein described is that it confirms previous conclusions by experiments on a larger scale, it explains the mechanisms responsible for the lack of reproductive capacity of females mated with hybrid males and also shows that the induced male sterility through interspecific hybridization is not irreversible. The fertility of male hybrids was partially restored when the progeny of hybrid females were backcrossed two additional generations to parental males. Fertility of hybrid females, as measured by the yield of adults capable to reproduce, indicate that the reproductive performance of these females decreased when their descendents were backcrossed additional generations to parental males.

MATERIAL AND METHODS

All insects used in this study were laboratory colonized from nymphs and/or adults brought in from the field in 1969-1970. *T. infestans* (Klug, 1834) and *T. sordida* (Stal, 1859) originated from the State of São Paulo (Fig. 1), where the former is domestic and the latter is mainly confined to primitively constructed chicken houses. *T. pseudomaculata* (Corrêa & Espinola, 1964), referred previously as *T. maculata*¹⁰ was collected in the State of Pernambuco where it is mainly peridomestic. *T. brasiliensis* (Neiva, 1911) originated in the State of

Ceará where it lives in burrows of small rodents and sporadically invades human huts. *R. prolixus* (Stal, 1859) and *R. neglectus* (Lent, 1954) were collected as nymphs in a birds' nest in the State of São Paulo. Maintenance of the above species and the interspecific mating procedures have been previously described¹⁰.

Series of multiple and single pairs of insects (Table 2) were set up from the stock cultures reared continuously as mixed generations, thus bearing more resemblance to field populations. Only data from single pairs were analyzed for two reasons; (1) the number of successful matings in mass pairing was unknown and (2) to avoid the crowding effect on reproductive performance of hybrids and of their descendents. Egg production by female progeny from interspecific crosses, by hybrid females backcrossed to parental males and by their female descendents, was recorded throughout the female life-span.

Interspecific parent crosses and descendents were designated P and P₁ respectively. Hybrid females backcrossed to parental males were designated BC₁. Female progeny of BC₁ backcrossed to parental males were referred BC₂. Females of BC₂ crossed with parental males were designated BC₃. Female progeny of BC₃ backcrossed to parental males were designated BC₄.

RESULTS

Before going into details about the results obtained, it would not be out of place to direct attention to the differences in biology of the species utilized in our experiments, with regard to development duration of immatures and adults, sex ratios, mating behaviour, longevity, fecundity and fertility in laboratory colonized populations (Table 1).

Triatominae are easy to rear, no special requirements as to mating behaviour, little loss of progeny, because most of the eggs hatch and develop in adults with a sex ratio, indicating a surplus of females in all *Triatoma* species tested.

The somewhat different sex ratio in *R. neglectus*, indicating a predominance of males is rather difficult to explain, though it might suggest a genus characteristic, the male frequency in *R. prolixus* species being also greater than in the *Triatoma* species. On the other hand it is noteworthy that although the 84.5% egg hatch in *R. neglectus* was comparable with that of *Triatoma* species (73-92.4%), adult emergence was rather low; 49% in *R. neglectus* vs. 72-89% in *Triatoma*. High losses of emerging adults in *R. neglectus* resulted from incomplete ecdysis. While emerging adults in *Triatoma* rarely die during transition from the 5th instar nymphs, in *R. neglectus* many adults were unable to shed the nymphal cuticle. Therefore it is tentatively concluded that deaths caused by incomplete ecdysis, occurred more frequently among females, thus increasing the yield of living males.

Parental crosses. — Progeny P₁ from *T. pseudomaculata* x *T. sordida* were obtained with either species furnishing the parent male or female (Table 2). However, the crosses of *T. pseudomaculata* female with *T. sordida* male were more frequently successful than the reciprocal crosses. Of the total pairs of *T. pseudomaculata* ♀ x *T. sordida* ♂ 95% produced progeny, but only 62% of reciprocal pairs did.

The *T. pseudomaculata* females mated with *T. sordida* males laid an average of 219 eggs vs. 269 per female in the reciprocal cross. *T. sordida* male seems to be superior to *T. pseudomaculata* male in fertilizing the female; 61% of eggs from *T. pseudomaculata* ♀ x *T. sordida* ♂ hatched vs. 49% from the reciprocal cross. However, there was no significant difference in the percentage of adults developed from hatched eggs in the reciprocal crosses, 36 and 40%, around half of the yield from intraspecific crosses.

The sex distribution in adult populations seems to be a characteristic affected by the father species. The well pronounced female predominance in *T. sordida* population (male/female-0.76) tended to increase in progeny from *T. pseudomaculata* ♀ x *T. sordida* ♂ (0.67). In the *T. pseudomaculata* populations

the sex ratio is almost evenly distributed (0.91) and it behaved similarly in hybrids from *T. sordida* ♀ x *T. pseudomaculata* ♂ (1.0).

Progeny were obtained from *T. pseudomaculata* ♀ x *T. infestans* ♂ but no descendents from the reciprocal cross. Of the total interspecific pairs set up 83% mated. Egg production was low, an average of 195 per females vs. the mean of 920 eggs laid by *T. infestans* females and 621 eggs produced by *T. pseudomaculata* females mated with their own males. Only 33% of these eggs hatched, approximately 1/3 of those in intraspecific crosses. Thirty percent of the hatched eggs reached the adult stage vs. 72 and 89% emerged adults from intraspecific matings in *T. pseudomaculata* and *T. infestans* populations respectively (Table 1). As for the sex distribution, there was a considerable predominance of female offspring (male/female-0.65), but approaching more closely the father species (0.81) than the mother species (0.91).

Progeny were also obtained from pairing *T. pseudomaculata* females with *T. brasiliensis* males, but no offspring from the reciprocal cross. Only 13% of the females reproduced and fecundity was low, the average number of eggs per female was 172 vs. the mean of 583 from intraspecific crosses of *T. brasiliensis*. Only 10% of these eggs hatched, but survival of the immature stages was rather high, 73% developed into adults vs. 86% developed from pairing *T. brasiliensis* female with her own male. Around 61% of descendents were females, the sex ratio being 0.60 vs. 0.91 in *T. pseudomaculata* population and 0.82 in the *T. brasiliensis* population (Table 1). Although the sex distribution differed from that of both parents it approached rather the father species, *T. brasiliensis*.

In the *Rhodnius* genus, progeny were obtained from the unilateral cross of *R. prolixus* female with *R. neglectus* male. Only 45% of the paired females reproduced. The average number of eggs per female was 476 vs. 915 eggs per *R. neglectus* female paired with her own male. Only 4.6 per cent of eggs hatched vs. 84% and 92% hatch in the *R. neglectus* and *R. prolixus* species respectively. Though hatcha-

bility was low, the survival of those that hatched and developed into adults was 53% vs. 45% and 68% in the *R. neglectus* and *R. prolixus* populations respectively. Approximately 43% among the progeny were females, thus the predominant sex in progeny being the male. The sex ratio was 1.33 vs. 1.15 in the *R. neglectus* population and 0.97 in the *R. prolixus* population. Here again the sex distribution, indicating a predominance of males, was more like in the father species *R. neglectus*.

The data obtained indicate hybrid weakness confined to the very young nymphs. It was not evidenced among adults, which as a rule demonstrated hybrid vigor. Early deaths occurred frequently after confinement of female hybrids with parental males, when separately caged they lived much longer, nonetheless the average life span for interspecific hybrid females was 216-312 days (Table 2) vs. 325-588 days for parent species (Table 1).

Adult hybrids more closely resemble one species than the other, indicating a dominance phenomenon, behaving similarly in reciprocal hybrids when both occurred. The two dominant characters color and size were retained in the offspring from backcrossing of hybrid females to parental males. As seen in Fig. 2, adults from *T. pseudomaculata* ♀ x *T. infestans* ♂ phenotypically resemble *T. infestans*, though the dark transverse bands of the connexum and the parallel light stripes on the pronotum are characters inherited from *T. pseudomaculata*. The external reproductive structures in the male hybrid derived from *T. infestans*, while the scutellum and the 9th and 10th tergite in the females resemble *T. pseudomaculata* structures. Hybrids from *T. pseudomaculata* ♀ x *T. brasiliensis* ♂ more closely resemble the large blond *T. brasiliensis* than the small dark *T. pseudomaculata*. Mixed characters of both parents are visible in the external reproductive structures of females and males. Hybrids, from *T. pseudomaculata* x *T. sordida* phenotypically resemble *T. sordida* by the color patterns of the wings, connexival band and legs. The dark head and the light parallel stripes on the pronotum are characters inherited from *T. pseudomaculata*. The external genitalia of both sexes represent a mixture of structural characters derived from both parents. Hybrids, from *R.*

Table 1 – Female life span, mating frequency, number of eggs/♀, development of eggs, percentage egg hatch, development of adults percentage adult emergence and sex ratio in laboratory reared triatominae.

| Species | Life spans (days) | Nº of spermatophores/♀ * | | Nº of eggs/♀ | Egg development (days) | | Percentage hatch | Adult development (days) | | Percentage of emerged adults | Sex ratio ♂/♀ |
|--------------------------|----------------------|--------------------------|-------|--------------------|---------------------------|--------------------|------------------|-----------------------------|--------------------|---------------------------------|------------------|
| | | avg. | Range | | min. & max. | optimum range** | | min. & max. | optimum range** | | |
| <i>T. pseudomaculata</i> | 538.3 ± 21.13 (12) | 11,2 | 7-15 | 621.2 ± 38.24 (12) | 7-27 | 16-22 | 77.3 (126-3978) | 100-310 | 114-144 | 72.0 (12-272) | 0.91 |
| <i>T. sordida</i> | 582.9 ± 35.49 (11) | | | 684.2 ± 37.64 (11) | 8-25 | 15-19 | 87.7 (127-4319) | 87-218 | 100-140 | 88.7 (8-275) | 0.76 |
| <i>T. infestans</i> | 488.6 ± 18.02 (7) | 25.4 | 17-39 | 919.7 ± 34.58 (7) | 8-21 | 15-19 | 92.4 (120-5576) | 90-205 | 105-145 | 88.8 (18-563) | 0.81 |
| <i>T. brasiliensis</i> | 325.5 ± 44.44 (11) | 28.0 | 21-34 | 582.9 ± 36.06 (11) | 15-31 | 18-24 | 92.1 (108-3714) | 106-254 | 120-150 | 85.8 (10-269) | 0.82 |
| <i>R. neglectus</i> | 588.2 ± 15.58 (8) | | | 914.7 ± 21.97 (8) | 9-23 | 13-15 | 84.5 (90-3658) | 84-360 | 138-193 | 44.8 (6-183) | 1.15 |
| <i>R. prolixus</i> | | 72.2 | 57-87 | | 7-15 | 13-14 | 91.8 (1-1044) | 82-360 | 95-135 | 68.4 (13-329) | 0.97 |

Standard deviation expressed as percentage of the mean (CV).

Single records in brackets indicate number of specimens, double records indicate number of batches and total specimens tested.

* Spermatophores recorded only for the first four months of oviposition.

** Highest percentage developed.

Table 2 — Fertility frequency, number of eggs, percentage egg hatch, percentage of adults emerged, sex frequency and sex ratio in interspecific crosses between vectors of Chagas' disease

| Type of cross P | N♀ fertile ♀♀ | Total* eggs | Percentage hatch | Percentage adults | P ₁ | | | P ₁ ♀♀ life span (days) Avg. & range |
|--------------------|---|----------------|---------------------|---|----------------|------------|--------------|--|
| | | | | | Sex frequency | | ♂/♀ ratio | |
| | | | | | ♀♀ | ♂♂ | | |
| pm ♀ x so ♂ | 19 (20) ^a (50) ^b | 4154 1056 | 60.6 47.9 | 36.0 (2519) ^c 33.5 (506) | 543 117 | 363 54 | 0.67 0.46 | 265.2 (12) 77-458 |
| pm ♀ x in ♂ | 15 (18) (39) | 2929 2163 | 33.0 15.0 | 29.7 (975) 18.5 (325) | 176 36 | 114 24 | 0.65 0.67 | 215.7 (9) 68-373 |
| pm ♀ x br ♂ | 2 (15) (30) | 344 1684 | 9.6 0.7 | 72.7 (33) 25.0 (12) | 15 2 | 9 1 | 0.60 0.50 | |
| pr ♀ x ne ♂ | 9 (20) | 4281 | 4.6 | 52.7 (199) | 45 | 60 | 1.33 | 312.1 (9) 258-405 |
| Reciprocal crosses | | | | | | | | |
| pm ♂ x so ♀ | 10 (16) (50) | 2695 2571 | 49.5 29.3 | 40.0 (1331) 31.5 (753) | 272 109 | 271 128 | 1.00 1.17 | 220.5 (10) 73-349 |
| pm ♂ x in ♀ | 0 (20) | 1860 | 0 | 0 | 0 | 0 | | |
| pm ♀ x br ♀ | 0 (27) | 3903 | 0 | 0 | 0 | 0 | | |
| pr ♂ x ne ♀ | 0 (18) | 1563 | 0 | 0 | 0 | 0 | | |

pm — *T. pseudomaculata*; so — *T. sordida*; in — *T. infestans*; br — *T. brasiliensis*; pr — *R. prolixus*; ne — *R. neglectus*.

* — Recorded only for fertilized females.

a — Total females in single-pair crosses

b — Number of fertile females not determined in multiple-pair crosses.

c — Number hatched eggs.

prolixus ♀ x *R. neglectus* ♂ are more like *R. prolixus*. The male claspers and the female VIII segment resemble *R. prolixus* structures. Structural characters of *R. neglectus* are shown by the length of the rostrum and the pigofor.

Male sterility — No progeny were obtained when male hybrids from interspecific crosses, were paired with parental females of either species (Table 3). However, successful matings, as measured by dropped spermatophores, occurred in 90% of the total of 65 pairs, set up for dissections and composed of females of either species and hybrid males, from *T. pseudomaculata* x *T. sordida*. All spermatophores were smeared with sperm. When the males were dissected the vesicula seminalis were found to be packed with spermatozoa, some normal looking and moving, others not developed and motionless. When the females were dissected all but two were found to have empty spermatecae. The two females with sperm in the spermatecae laid a few embryonated eggs that did not hatch. Females paired with hybrid males, from *T. pseudomaculata* ♀ x *T. infestans* ♂ and those mated to males from *T. pseudomaculata* ♀ x *T. brasiliensis* ♂ behaved similarly. Copulation had occurred, single and sometimes multiple spermatophores smeared with sperm were discharged, but no spermatozoa were found in the spermatecae. Only one female of the 10 paired with male hybrids from *T. pseudomaculata* ♀ x *T. infestans* ♂ demonstrated spermatozoa in the spermatecae and several dead embryonated eggs. Similarly behaved one of the 10 females paired with male hybrids from *T. pseudomaculata* ♀ x *T. brasiliensis* ♂. It seems therefore that lack of progeny from crosses of hybrid males to parental females was primarily due to the absence of sperm in the spermatecae of females.

Fertility of female hybrids. — Adult progeny were obtained from backcrosses of any type of female hybrids with parental males of either species (Table 3). However, the overall reproductive performance of the female hybrids was rather poor. Neither male elicited a normal oviposition response from either type of hybrid female, as measured by the number of eggs laid. Hatchability was very low, ranging from 0.8 to 6.8%, adult emergence was poor and female frequency was above normal in all *Triatoma* hybrids.

The reproductive potential of the female hybrid differed greatly, depending primarily on the parental cross, in case of reciprocal pairing, and also on the parental male with which it was paired. For example, female hybrids, from *T. pseudomaculata* ♀ x *T. sordida* ♂ mated more frequently with *T. sordida* males than females from the reciprocal cross, *T. pseudomaculata* ♂ x *T. sordida* ♀ mated with the same type of males (56% vs. 67%), laid more eggs (180 vs. 133), produced more adults (36% vs. 26%), with a lesser predominance of females (male/female ratio, 0.74 vs. 0.64). However, female fertility was lower in the former backcross, egg hatch being 5.2% vs. 6.5% in the latter. The *T. sordida* male was superior to *T. pseudomaculata* male in mating capability (67-86 vs. 60-61%) and in furnishing less disturbed sex ratios (0.64-0.74 vs. 0.38-0.62). The reproductive characteristics of the female hybrid from *T. pseudomaculata* ♀ x *T. infestans* ♂ differed depending upon the parental male with which she was paired, whether it was of the mother species or father species. For example, the *T. infestans* male appeared to be superior to *T. pseudomaculata* male in eliciting better ovipositional response (544 eggs per female vs. 288), higher percentage of adult progeny (92% vs. 53%) and less abnormal sex ratio (0.71 vs. 0.44). The hybrid female, from *R. neglectus* ♀ x *R. prolixus* ♂ mated readily with *R. prolixus* male (90%), produced a high egg complement (448 per female) and an adult progeny with an almost normal sex distribution. Fertility measured by egg hatchability was low, only 2.9% of eggs hatched.

Fertility of backcross progeny from interspecific crosses. — Backcrossing of hybrid females to parental males improved female fertility by the 2nd, 3rd and 4th generation, as measured by egg hatchability (Table 4). However, the increased egg hatch differed greatly depending on the male species with which the female hybrid and her female descendants were paired. For example, egg hatch increased from 5% in BC₁ to 62-50% in BC₃ and BC₄ respectively, in backcrossing of the hybrid female, from *T. pseudomaculata* x *T. sordida* and her descendants with *T. sordida* male. But only 2-3% of egg hatched when the same type of hybrid female and her female progeny were backcrossed 3 and 4 additional generations (BC₃, BC₄) with *T. pseudoma-*

Table 3 — Interspecific hybrids backcrossed to parents for proof of sterility.

| Type of cross P | Type of backcross BC ₁ | Nº fertile ♀♀ | Percentage hatch | Percentage adults | F ₁ from BC ₁ | | |
|--------------------|--------------------------------------|---------------------|-------------------------|-------------------------|-------------------------------------|----|-----------|
| | | | | | ♀♀ | ♂♂ | ♂/♀ ratio |
| pm ♀ x so ♂ | P ₁ ♀ x pm ♂ | 8 (13) ^a | 5.4 (1455) ^b | 49.3 (79) ^c | 24 | 15 | 0.62 |
| | p ₁ ♀ x so ♂ | 12 (14) | 5.2 (2157) | 35.7 (112) | 23 | 17 | 0.74 |
| | P ₁ ♂ x pm ♀ | 0 (19) | 0 (2184) | 0 | 0 | 0 | |
| | P ₁ ♂ x so ♀ | 0 (15) | 0 (1879) | 0 | 0 | 0 | |
| pm ♂ so ♀ | P ₁ ♀ x so ♂ | 8 (12) | 6.5 (1068) | 26.1 (69) | 11 | 7 | 0.64 |
| | P ₁ ♀ x pm ♂ | 9 (15) | 6.8 (902) | 18.0 (61) | 8 | 3 | 0.38 |
| | P ₁ ♂ x so ♀ | 0 (25) | 0 (3472) | 0 | 0 | 0 | |
| | P ₁ ♂ x pm ♀ | 0 (25) | 0 (1673) | 0 | 0 | 0 | |
| pm ♀ x in ♂ | P ₁ ♀ x in ♂ | 3 (10) | 0.8 (1633) | 92.3 (13) | 7 | 5 | 0.71 |
| | P ₁ ♀ x pm ♂ | 6 (10) | 2.5 (1733) | 53.5 (43) | 16 | 7 | 0.44 |
| | P ₁ ♂ x in ♀ | 0 (15) | 0 (819) | 0 | 0 | 0 | |
| | P ₁ ♂ x pm ♀ | 0 (15) | 0 (1537) | 0 | 0 | 0 | |
| pr ♀ x ne ♂ | P ₁ ♀ x pr ♂ | 9 (10) | 2.9 (4031) | 34.2 (117) | 21 | 19 | 0.90 |
| | P ₁ ♂ x pr ♀ | 0 (10) | 0 (2048) | 0 | 0 | 0 | |

a — Total females tested in brackets.

b — Total eggs in brackets. For hybrid females, eggs recorded only for fertilized individuals.

c — Total eggs hatched in brackets.

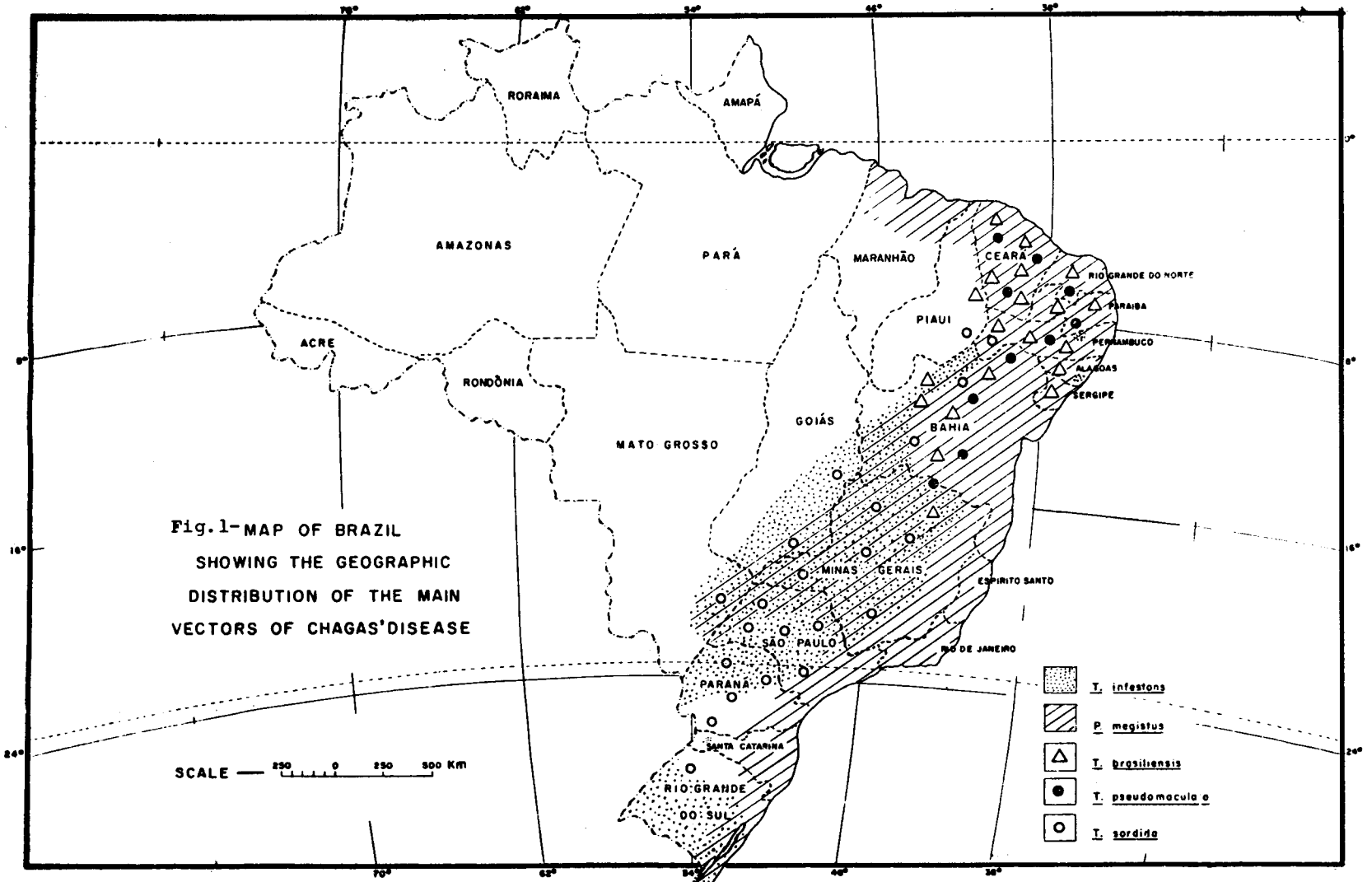


Fig.1-MAP OF BRAZIL
SHOWING THE GEOGRAPHIC
DISTRIBUTION OF THE MAIN
VECTORS OF CHAGAS' DISEASE

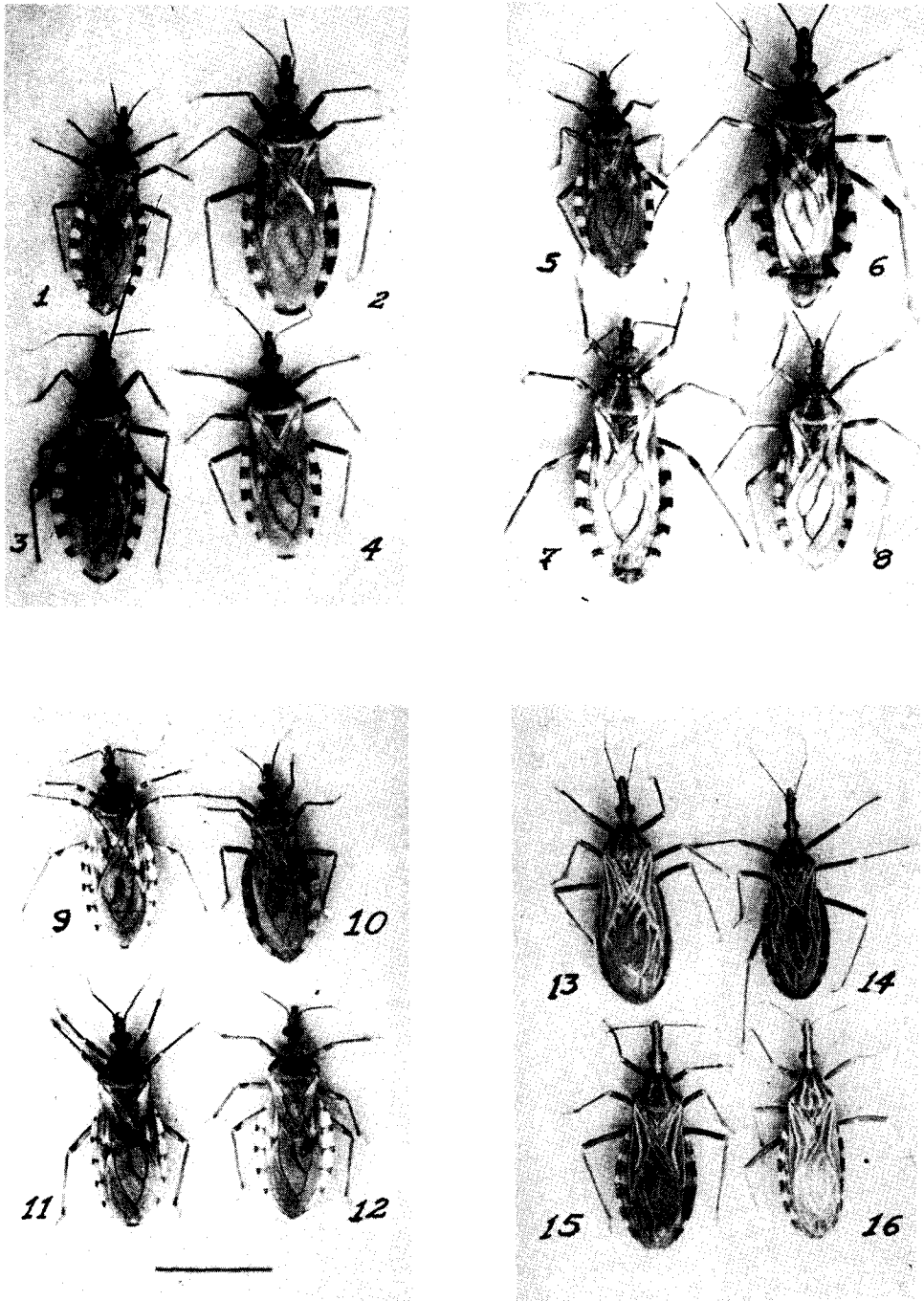


Fig. 2 — Showing wild species of Triatominae and hybrids resulted from interspecific crosses:

- 1, 2 — *T. pseudomaculata* ♀ x *T. infestans* ♂, 3, 4 — ♀ & ♂ hybrids:
 5, 6 — *T. pseudomaculata* ♀ x *T. brasiliensis* ♂, 7, 8 — ♂ & ♀ hybrids:
 9, 10 — *T. sordida* ♂ x *T. pseudomaculata* ♀, 11, 12 — ♂ & ♀ hybrids:
 13, 14 — *R. prolixus* ♀ x *R. neglectus* ♂, 15, 16 — ♀ & ♂ hybrids:

Table 4 – Fertility frequency, percentage hatch, percentage of adults emerged, sex frequency and sex ratio when females from successive backcrosses were paired with parent males.

| Type of cross P | Type of backcrosses BC | N♀ fertile ♀♀ | Percentage hatch* % | Percentage adults % | Sex frequency | | ♂/♀ ratio |
|--|--|---|-------------------------|-------------------------|---------------|----|-----------|
| | | | | | ♀♀ | ♂♂ | |
| pm ♀ x so ♂ | P ₁ ♀ x so ♂ BC ₁ | 12 (14) ^a | 5.2 (2157) ^b | 35.7 (112) ^c | 23 | 17 | 0.74 |
| | BC ₁ ♀ x so ♂ BC ₂ | 9 (14) | 21.5 (1323) | 14.8 (284) | 23 | 19 | 0.83 |
| | BC ₂ ♀ x so ♂ BC ₃ | 8 (8) | 62.4 (1191) | 1.2 (743) | ** | ** | ** |
| | BC ₃ ♀ x so ♂ BC ₄ | 2 (2) | 50.4 (117) | ** | ** | ** | ** |
| | P ₁ ♀ x pm ♂ BC ₁ | 8 (13) | 5.4 (1455) | 49.3 (79) | 24 | 15 | 0.62 |
| | BC ₁ ♀ x pm ♂ BC ₂ | 8 (8) | 15.9 (2077) | 52.9 (232) | 99 | 76 | 0.77 |
| | BC ₂ ♀ x pm ♂ BC ₃ | 2 (5) | 3.0 (491) | 60.0 (15) | 5 | 4 | 0.80 |
| | BC ₃ ♀ x pm ♂ BC ₄ | 1 (3) | 2.2 (231) | ** | ** | ** | ** |
| | P ₁ ♀ x so ♂ BC ₁ | 12 (14) | 5.2 (2157) | 35.7 (112) | 23 | 17 | 0.74 |
| | BC ₁ ♀ x pm ♂ BC ₂ | 5 (5) | 11.9 (1359) | 44.0 (162) | 34 | 37 | 1.09 |
| | BC ₂ ♀ x pm ♂ BC ₃ | 2 (2) | 25.8 (996) | 18.3 (257) | 32 | 15 | 0.47 |
| | BC ₃ ♀ x pm ♂ BC ₄ | 1 (5) | 20.3 (227) | ** | ** | ** | ** |
| pm ♂ x so ♀ | P ₁ ♀ x so ♂ BC ₁ | 8 (12) | 6.5 (1068) | 26.1 (69) | 11 | 7 | 0.64 |
| | BC ₁ ♀ x so ♂ BC ₂ | 1 (3) | 45.2 (126) | 89.5 (57) | 27 | 24 | 0.90 |
| | BC ₂ ♀ x so ♂ BC ₃ | 5 (5) | 53.3 (477) | 9.0 (254) | 11 | 12 | 1.09 |
| | P ₁ ♀ x pm ♂ BC ₁ | 9 (15) | 6.8 (902) | 18.0 (61) | 8 | 3 | 0.38 |
| | BC ₁ ♀ x so ♂ BC ₂ | 2 (2) | 11.4 (536) | 70.5 (61) | 26 | 17 | 0.65 |
| | pm ♀ x in ♂ | P ₁ ♀ x pm ♂ BC ₁ | 6 (10) | 2.5 (1733) | 53.5 (43) | 16 | 7 |
| BC ₁ ♀ x pm ♂ BC ₂ | | 3 (13) | 2.1 (1702) | 30.5 (36) | 6 | 5 | 0.87 |
| BC ₂ ♀ x pm ♂ BC ₃ | | 2 (2) | 8.9 (67) | ** | ** | ** | ** |
| P ₁ ♀ x in ♂ BC ₁ | | 3 (10) | 0.8 (1633) | 92.3 (13) | 7 | 5 | 0.71 |
| BC ₁ ♀ x in ♂ BC ₂ | | 1 (4) | 0.1 (723) | ** | ** | ** | ** |
| P ₁ ♀ x pm ♂ BC ₁ | | 6 (10) | 2.5 (1733) | 53 (43) | 16 | 7 | 0.44 |
| BC ₁ ♀ x in ♂ BC ₂ | | 4 (5) | 0.7 (1446) | 20 (10) | 1 | 1 | 1.00 |
| P ₁ ♀ x in ♂ BC ₁ | | 3 (10) | 0.8 (1633) | 92.3 (13) | 7 | 5 | 0.71 |
| BC ₁ ♀ x pm ♂ BC ₂ | | 1 (1) | 0.3 (997) | 33.3 (3) | 1 | 0 | |
| pr ♀ x ne ♂ | | P ₁ ♀ x pr ♂ BC ₁ | 9 (10) | 2.9 (4031) | 34.2 (117) | 21 | 19 |
| | BC ₁ ♀ x pr ♂ BC ₂ | 4 (5) | 16.4 (1574) | 10.8 (258) | 15 | 13 | 0.85 |

* Eggs recorded only in fertilized females
 a Total females in brackets
 b Total eggs in brackets
 c Total eggs hatched in brackets
 ** Under observation

Table 5 — Partial restoration of fertility in hybrid males through backcrossing of hybrid females to parent males

| Origin of progeny | No. fertile ♀ ♀ | Percentage hatch | Percentage adults | Sex frequency | | ♂/♀ ratio |
|--|---------------------|-----------------------|-----------------------|------------------|-----|--------------|
| | | | | ♀ ♀ | ♂ ♂ | |
| Paired progeny (P ₁) from interspecific crosses (P) | | | | | | |
| pm ♀ x so ♂ | 0 (15) ^a | 3 (1138) ^b | 7 | 2 | 2 | - |
| so ♀ x pm ♂ | 0 (13) | 0 (725) | 0 | 0 | 0 | - |
| pm ♀ x in ♂ | 0 (19) | 0 (2849) | 0 | 0 | 0 | - |
| pr ♀ x ne ♂ | 0 (8) | 0 (815) | 0 | 0 | 0 | - |
| Paired progeny (F ₁) from 1st backcross (BC ₁) | | | | | | |
| ♀ (pm ♀ x so ♂) x so ♂ | 0 (7) | 0 (976) | 0 | 0 | 0 | - |
| ♀ (pm ♀ x so ♂) x pm ♂ | 0 (4) | 0 (733) | 0 | 0 | 0 | - |
| ♀ (so ♀ x pm ♂) x so ♂ | 0 (6) | 0 (1543) | 0 | 0 | 0 | - |
| ♀ (so ♀ x pm ♂) x pm ♂ | 0 (3) | 0 (878) | 0 | 0 | 0 | - |
| ♀ (pm ♀ x in ♂) x pm ♂ | 0 (4) | 0 (1154) | 0 | 0 | 0 | - |
| ♀ (pm ♀ x in ♂) x in ♂ | 0 (3) | 0 (647) | 0 | 0 | 0 | - |
| ♀ (pr ♀ x ne ♂) x pr ♂ | 0 (6) | 0 (1257) | 0 | 0 | 0 | - |
| Paired progeny (F ₁) from 2nd backcross (BC ₂) | | | | | | |
| ♀ (pm ♀ x so ♂) x so ♂ x so ♂ | 8 (20) | 73.6 (1474) | * (1086) ^c | 5 | 3 | |
| ♀ (pm ♀ x so ♂) x pm ♂ x pm ♂ | 4 (10) | 12.4 (960) | 17.6 (119) | 11 | 10 | 0.90 |
| ♀ (so ♀ x pm ♂) x so ♂ x so ♂ | 6 (13) | 50.4 (2284) | 5.4 (1152) | 35 | 28 | 0.80 |
| ♀ (pr ♀ x ne ♂) x pr ♂ x pr ♂ | 8 (9) | 18.2 (2932) | 24.0 (533) | 51 | 77 | 1.51 |
| Paired descendants (F ₂) from F ₁ of 2nd backcross (BC ₂) | | | | | | |
| F ₂ from ♀ (pm ♀ x so ♂) x pm ♂ x pm ♂ | 4 (7) | 41.8 (529) | * (221) | 6 | 4 | |
| F ₂ from ♀ (so ♀ x pm ♂) x so ♂ x so ♂ | 7 (8) | 40.9 (1964) | 4.2 (804) | 20 | 14 | 0.70 |

The way applied to present section 3 & 4 of column 1 was convenient but can be confusing; it does not indicate that the hybrid female was twice crossed with the parental male, it was crossed once only (BC₁) and the female progeny of this cross was again paired with the male of the same type (BC₂).

a - Total females in brackets

b - Total eggs in brackets

c - Total eggs hatched in brackets

* - Under observation

culata male. The superiority of *T. sordida* male over *T. pseudomaculata* male in increasing egg hatchability was also demonstrated in backcrossings of hybrid females and their descendents with males of mixed parent species. For example, when the hybrid female from *T. pseudomaculata* ♀ x *T. sordida* ♂ was backcrossed with *T. sordida* male and her female progeny were paired for 3 or 4 generations with *T. pseudomaculata* male, egg hatch was not as high as it was when only *T. sordida* male was used, 20-26% hatch in the former BC₃ and BC₄ vs. 50-62% in the latter. Nevertheless, egg hatch was 8-9 times greater than that recorded in BC₃ and BC₄ of backcrossing with *T. pseudomaculata* male exclusively. However, improved hatchability does not indicate improved survival of progeny that hatched. No detailed examination of the embryos exist, but what exists suggest that by sustained backcrossing of the hybrid female and her descendents with *T. sordida* male, death of progeny was postponed to the later stages. There was less mortalities before hatching but nymphs that hatched died abundantly, thus decreasing considerably the proportion of developed adults. The contrary occurred in backcrossings of the hybrid female and her progeny with *T. pseudomaculata* male, egg hatchability was very poor, but around 60% of eggs hatched developed into adults. No improvement of reproductive performance occurred in backcrossing of the hybrid female from the unilateral cross of *T. pseudomaculata* with *T. infestans*. Interestingly, the egg complement in BC₂ females was unusual high (range, 289-997/♀), and so was also their life-span, up to 20 months. Nevertheless, egg hatchability was low and tended to decrease, it ranged 0.1-2.1% in BC₂ vs. 0.8-2.5 in BC₁.

In the *Rhodnius* genus egg hatchability increased when BC₁ females were again backcrossed with *R. prolixus* male (16% in BC₂ vs. 3% in BC₁) but, mortalities also increased in immature forms. Thus the yield of adults in BC₂ from *R. neglectus* ♂ x *R. prolixus* ♀ was only 11% vs. 34% in BC₁. Nevertheless the sex distribution in the two populations was alike as indicated by the male/female ratio, 0.90 and 0.85 (Table 4).

As shown in Table 5, no progeny were obtained when hybrid males and hybrid females were paired, due to sterility of the male hybrid. Similarly no progeny were obtained when descendents (BC₁) of hybrid females backcrossed to parental males, were paired, because they behaved like their maternal pa-

rent, in that only hybrid females were fertile. However successful mating occurred among progeny of BC₂. Over 40% of BC₂ descendents, from *T. pseudomaculata* x *T. sordida* backcrossed to *T. sordida* male, mated successfully and were fertile as indicated by egg hatchability ranging from 50.4 to 73.6%. When hybrid females from this cross were backcrossed for two additional generations with *T. pseudomaculata* male, again 40% of BC₂ progeny mated successfully, but only 12.4% of eggs hatched. Nevertheless, the yield of adults increased, it was 17.6% vs. 5.4% from crossing of BC₂ progeny derived from backcrossing with *T. sordida* male. Interestingly the sex distribution in the populations from paired progeny (F₁) of BC₂ approached very closely that of the parent species, *T. sordida* and *T. pseudomaculata*, showing the characteristic for *Triatoma* species 10 to 20% predominance of females, male/female ratio was 0.80 and 0.90.

An interesting aspect of the partially restored male fertility is the fact that over 50% of F₂ descendents originated from the 2nd backcross of hybrid females from *T. pseudomaculata* x *T. sordida*, mated successfully, thus furnishing a 3rd generation of BC₂ adults.

Successful mating occurred also between progeny of BC₂ from *R. prolixus* ♀ x *R. neglectus* ♂. Eight of nine paired progeny produced 18.2% hatched eggs, 24% of which developed into adults. Thus fertility of BC₂ male progeny was partially restored when the BC₁ females were paired with *R. prolixus* male.

COMMENTS

The interspecific crosses between *Triatoma* results in male sterility as shown by lack of progeny from backcrossing of hybrid males to parental females. The male descendents from BC₁ were also unable to engender living offspring from parental females. The obvious reason for the lack of progeny was the absence of sperm in the spermatocae of parent females mated with hybrid males. But sperm were transferred in mating, since spermatophores dropped by mated females were smeared with spermatozoa, some of normal appearance, others underdeveloped and motionless. Thus sperm for some reason were unable to reach the spermatocae. Davey⁴ observed that the removal of the opaque accessory glands in *R. prolixus* prevents the normal migration of the sperm in female which is inseminated by it, and concluded that "the migration is a result of

characteristic rhythmic contractions set up in the oviduct by the opaque accessory secretion of the male". It would therefore appear that the inability of sperm to reach the spermathecae of the female, mated with the hybrid male, is due primarily to failure on the part of hybrid males to produce and/or to incorporate male accessory secretions in the spermatophore bulb. Whether the sperm of the hybrid male, which were unable to reach the spermathecae of the female mated with it, retained fertilizing capacity is not known. The fact, that a few of the females contained spermatozoa in the spermathecae and laid a few embryonated eggs that did not hatch indicate sperm deficiencies, that prevent the zygote from developing. This could be caused by chromosomal aberrations found in males from interspecific crosses. Schreiber et al.^{14, 15}, observing in hybrids from interspecific crosses a variety of spermatids with n , $2n$ and $3n$ chromosomes, that failed to develop into normal spermatozoa, tentatively concluded that hybrid sperm were not functional. This however remains to be checked by induced insemination. Presumably, similar factors were involved in the sterility of male progeny from the first backcross (BC_1) of hybrid females (P_1) with parental males, at least exactly the same type of chromosomal aberrations were reported by Schreiber et al.¹⁶ for both, the male resulted from the interspecific cross and for the male progeny of BC_1 .

However, the sterility induced through interspecific hybridization of Triatominae is not irreversible in nature, because backcrossing of hybrid females to parental males partially restored the fertility of males by the 3rd generation.

Hybrid females were less adversely affected than the hybrid male, nevertheless reproductive characteristics, such as fecundity, fertility (scored by egg hatchability) and adult emergence were highly reduced in female hybrids, successfully mated with parental males. The poor reproductive performance revealed by all hybrid females backcrossed with parental males could be tentatively explained by chromosomal aberrations, but cytological observations, as referred to the female hybrid from interspecific crosses of Triatominae, practically do not exist. Nevertheless, here again fertility was gradually improved backcrossing of the hybrid females and the female descendents of these females to parental males, the speed of recovery depending on the male species with which it was crossed. So far there is nothing that would explain why *T.*

sordida male was more efficient in restoring fertility of the hybrid female from the reciprocal cross, than *T. pseudomaculata* male. Similarly fertility of the female hybrid, from the unilateral cross between *T. pseudomaculata* female and *T. infestans* male, tended to increase when crossed with the mother species, and decreased when mated with the father species. But egg hatch is rather a poor criterion for scoring of hybrid fertility, the mere presence of hatched eggs does not indicate good reproductive performance. Fertility, measured by the yield of adults capable to reproduce, indicate that reproductive performance of hybrid females frequently decreased by continuous crossing with parental males, because increased hatchability was followed by increased mortalities among the immature forms. In other words, deaths of hybrid offspring were postponed to a later developmental stage.

Results obtained are rather discouraging, sterility as exhibited by the male hybrids from interspecific crosses did not present us with a potential method of genetic control to complement the conventional insecticidal technique, used in the control against the vector of Chagas' disease. The failure of the sperm to be transferred to the spermathecae, as shown in our experiments, indicates a prezygotic post-copulation incompatibility, thus making the hybrid males unsuitable in limiting the growth of wild populations into which they are meant to be released. Theoretically, it appears that hybrid females, due to the poor reproductive performance when mated with wild males, could be used for population suppression, if they compete efficiently with the wild population, this however remains to be seen.

Analysis of our crossing results permits an insight into the degree of genetic affinity between morphologically distinct species, and also reveals interspecific relationships of phenotypic dominance. *Triatoma* species, *infestans*, *sordida* and *brasiliensis*, showing perfect reproductive isolation among each other, could be linked to *pseudomaculata* which was found to cross with all three, though in varying degrees. The reciprocal crosses between *T. pseudomaculata* and *T. sordida* argues for a close relationship, while the unilateral crosses between *T. pseudomaculata* and the other species indicate a more distant relationship. Nevertheless, reproductive isolation barriers exist between all of them as evidenced by hybrid male sterility (presumably of ethiological nature) and by the

poor fertility in hybrid females crossed with parental males. Thus gene flow was very reduced but not absent, indicating that none of the four *Triatoma* species achieved full reproductive isolation.

Genetic affinities were also revealed between two *Rhodnius* species, as evidenced by adult progeny developed from crossing *R. prolixus* female with *R. neglectus* male. Since in no instance was there possible to establish an F_1 population by crossing P_1 progeny, results obtained by no means suggest conspecificity, as Carvalheiros & Barreto³, analyzing our results, implies they do. The interspecific crosses rather provide evidence as to the probable existence of sterility barriers in nature. It has been also repeatedly pointed out in the literature on evolution and speciation within complexes of *Triatominae*^{1,8} that evidence of hybridization in the laboratory is not evidence of conspecificity. In spite of positive crossings between species they are usually kept apart in nature by ethiological, ecological and/or geographical barriers.

Carvalheiros & Barreto observing lack of progeny from crossings of *R. prolixus* female with *R. neglectus* male expressed some doubts as for the *R. prolixus* used in our study being "realmente *R. prolixus*". No direct evidence is available from the study of the authors that justified such a statement, nor the arguments used in support of it are convincing. It seems appropriate to mention that material utilized in our study originated from the State of São Paulo, Brazil and was collected in a birds' nest as a group of immature *R. neglectus* forms.

Upon emergence of adults they were readily distinguished as a mixed population of *R. prolixus* and *R. neglectus*^{1,7}. Since then colonies of both species are maintained separately. No prior information exist about the presence of *R. prolixus* in the State of São Paulo. Nevertheless, lack of exact information that would explain involuntarily or even voluntarily conditions that might contribute to the revealance of *R. prolixus* in a State where it has not been found in the past, does not invalidate the status of the population, as identified by the Entomologist of this Laboratory, A.B. Galvão and more recently by Dr. H. Lent.

R. prolixus utilized by the authors originated from Venezuela where it is domestic, *R. neglectus* derived from the State of São Paulo, Brazil where it was collected in palm trees. Evidently the perfect geographical and ecological isolating mechanisms prevented any gene exchange between this species colonized separately for many years in the laboratory. Therefore, it seems that the results described by the authors are more likely to be the result of a geographic isolation rather than a general reproductive isolation.

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RESUMO

O desenvolvimento de métodos integrados que indicam a libertação de machos estéreis para suplementar as técnicas convencionais com inseticidas, utilizadas no controle de insetos de importância médica, coloca a questão de os vetores da Doença de Chagas possuírem mecanismos naturais pela manipulação dos quais possam os mesmos serem controlados.

Resultados de experiências anteriores, previamente publicados, restringiram-se a informações fragmentares, levando várias questões, cujas respostas tornaram-se viáveis neste estudo.

*Híbridos interespecíficos originaram-se de cruzamentos recíprocos entre *T. pseudomaculata* e *T. sordida* e de cruzamentos unilaterais entre fêmeas *T. pseudomaculata* e machos *T. infestans*. Essas fêmeas cruzaram facilmente, revelando, porém, fecundidade diminuída, ainda que a progênie fosse relativamente abundante. Quando fêmeas *T. pseudomaculata* foram acasaladas com machos *T. brasiliensis*, a hibridação foi mais difícil, porque poucas das fêmeas cruzaram e aquelas que o fizeram tiveram sua fertilidade altamente reduzida. De todos os cruzamentos resultaram adultos, com a razão macho/fêmea indicando sensível excesso de fêmeas em todas as populações, exceto em uma.*

*O cruzamento entre as duas espécies de *Rhodnius* só se mostrou viável, quando a*

fêmea foi prolixus, sendo o macho neglectus. Essas fêmeas revelaram fecundidade razoável e fertilidade altamente reduzida, porém, mais de 50% dos ovos eclodidos evoluíram até adultos vigorosos, os machos predominando as fêmeas.

Os machos híbridos provenientes dos cruzamentos interespecíficos, tanto dos bilaterais quanto dos unilaterais, não chegaram a dar progênie, quando retrocruzados com fêmeas das espécies paternas, embora novos de espermatozoides, alguns de aspecto e movimento normal, outros imaturos e imóveis, fossem encontrados em suas vesículas seminais. Ainda que nenhuma inseminação artificial fosse realizada, o esperma em si não parece ser responsável pela esterilidade induzida através da hibridação interespecífica. Fêmeas acasaladas com esses híbridos, sem dúvida cruzaram, como ficou evidenciado pela rejeição de espermatozoides com vestígios de esperma; entretanto, suas espermatecas não continham espermatozoides. A incapacidade de espermatozoides migrarem para as espermatecas indica incompatibilidade pós-copulativa, pré-zigótica. Assim sendo, o macho híbrido não poderá competir com o macho natural e, conseqüentemente, não será útil na supressão das populações naturais.

As fêmeas híbridas facilmente cruzam com machos paternos, mostrando, porém, fertilidade dramaticamente reduzida, sendo seus filhos estéreis, como o foram aqueles de suas filhas férteis. Entretanto, os retrocruzamentos dessas fêmeas híbridas e das suas filhas com machos paternos restabelecem parcialmente a fertilidade de machos e aumentam sensivelmente a fertilidade das fêmeas. Todavia, adotando-se como critério para caracterizar a fertilidade de fêmeas, o número de adultos evoluídos, capazes de se reproduzir, em vez do número de ovos eclodidos, concluiu-se que o potencial reprodutivo das fêmeas híbridas vem diminuindo nos retrocruzamentos sucessivos com machos paternos. Portanto, sugere-se tentativamente que a libertação de fêmeas híbridas possa talvez suprimir a população natural caso essas atraíam eficientemente o macho.

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