INVOLVEMENT OF THE X-CHROMOSOME IN HYBRID MALE STERILITY FROM CROSSES BETWEEN SPECIES A AND SPECIES B OF THE TAXON *ANOPHELES DIRUS*[1, 2]

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ABSTRACT. A test for X chromosome involvement in causing hybrid male sterility was carried out with X chromosomes which in the salivary glands have distinctive polytene banding patterns designated as types X_A and X_B. *Anopheles dirus* species A and species B were mated with each other and the F_1 females backcrossed to each parental population. The males from the backcross to species B were found to include a significantly higher proportion which could inseminate than those from the other backcross. A difference in this direction is unexpected on the X–Y interaction hypothesis of the causation of the sterility. The results rule out such a hypothesis but suggest that the X chromosome may play a contributing role in causing sterility.

INTRODUCTION

The demonstration of male sterility in F_1 interspecific hybrids is well documented in several species of insects (Davidson 1974) and acarine ticks (Cwilich and Hadai 1963, Thompson et al. 1981). Such sterility has also been repeatedly reported in *Anopheles* species complexes (Kitzmiller 1967). That sterility of the males is due to defects in spermatogenesis has also been shown for the *An. gambiae* complex by Davidson et al. (1967) and Curtis (1978). However, the mechanism of hybrid sterility produced by interspecific crosses has been relatively little studied in anopheline species complexes.

The *Anopheles balabacensis* complex is now known to consist of at least 3 and possibly up to 6 cryptic species differentiated mainly by salivary polytene chromosome banding patterns (Hii 1984, in press) and mitotic and meiotic karyotypes (Baimai et al. 1981). Crosses between any pair of the 3 cryptic species are possible by forced mating and give various forms of hybrid male sterility (Hii 1984, in press). For example, when *An. dirus* species B ♂ is crossed to *An. dirus* species A[4] ♀, spermatogenesis in the F_1 is interrupted and sterility is 100%, but from the reciprocal cross normal-appearing spermatozoa are produced and when the F_1 males are mated to the F_1 famales, 50.5% of the resulting eggs hatch. Such a difference between the results of reciprocal crosses suggests that the X and/or X–Y chromosome are involved in causing the sterility. The female hybrids are fertile in backcrosses to each of the parental species and therefore a genetic study of the causation of the male sterility is possible by this route.

Accordingly, a preliminary test for X or Y chromosome involvement in causing hybrid male sterility was designed. As no marker genes are available in any of the 3 species of the *An. balabacensis* complex, the test was based on cytological observations of the X-chromosomes which can be easily distinguished in the larval salivary glands.

MATERIALS AND METHODS

The following colonies were used:

1. Species A: Bangkok/A—derived from a subcolony held at the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, USA and provided by Dr. R. W. Gwadz. It was originally called the Bangkok colony strain collected in 1964 at Khao Mai Khaeo, Chon Buri Province, Thailand (Esah and Scanlon 1966).

2. Species B: PERLIS/B—from a colony held at the Institute for Medical Research, Kuala Lumpur, Malaysia and originating from wild females collected in North Perlis in 1967.

The details of insectary conditions and maintenance are given by Curtis and Chalkley (1979). Crosses were made according to the following stepwise procedures:

Cross 1: *Anopheles dirus* species A males were crossed to species B females using the forced mating technique of Baker et al. (1962). Blood

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[4] The taxon *Anopheles dirus* Peyton and Harrison is considered as species A.

meals were provided from anaesthesized guinea pigs and glucose solution was always available.

Cross 2: Female hybrids from cross 1 were backcrossed to each of the parental species using the forced mating technique; F_1 males were discarded.

Cross 3: the resulting males from cross 2 were individually crossed to bloodfed females of species B using the induced mating technique again. Not more than 2 females were mated to each male. For each singly mated female, the testes of the corresponding male partner were dissected in saline solution and examined for the presence of spermatozoa. After mating, each female was isolated in an individual shell vial (75 × 2.5 cm) covered with a piece of mosquito netting and allowed to oviposit on a damp filter paper disc. The number of eggs and first instar larvae were counted and the spermathecae of ovipositing females were checked for the presence of spermatozoa. Egg batches were individually reared to the fourth stage in bowls. The salivary gland polytene chromosomes from 5 to 10 larvae from the family of each female were examined and the presence or absence of X-chromosome asynapsis determined using the techniques described by Kanda (1979).

RESULTS

Figure 1 shows the result of cross and backcrosses carried out with *An. dirus* A and B. The results of the first backcrosses (BC–A and BC–B) are presented in Table 1. A total of 69 and 75 crosses were made in backcrosses BC–A and BC–B respectively had mature sperm in their testes (Table 1) and spermathecal dissections and egg hatch tests gave results conforming approximately with these proportions. The males from backcross BC–A were found to include a significantly higher proportion which could inseminate than those from backcross BC–B ($X^2_1 = 4.5$, $0.01 < p < 0.05$).

Backcrosses BC–A and BC–B produced 16 and 14 viable egg hatches respectively (Fig. 1). On examination of the polytene chromosomes of the female larvae from backcross BC–A, 13 families showed a typical *dirus* X_B and a typical *dirus* X_A chromosome lying almost wholly unsynapsed as shown in Fig. 2, whereas there were

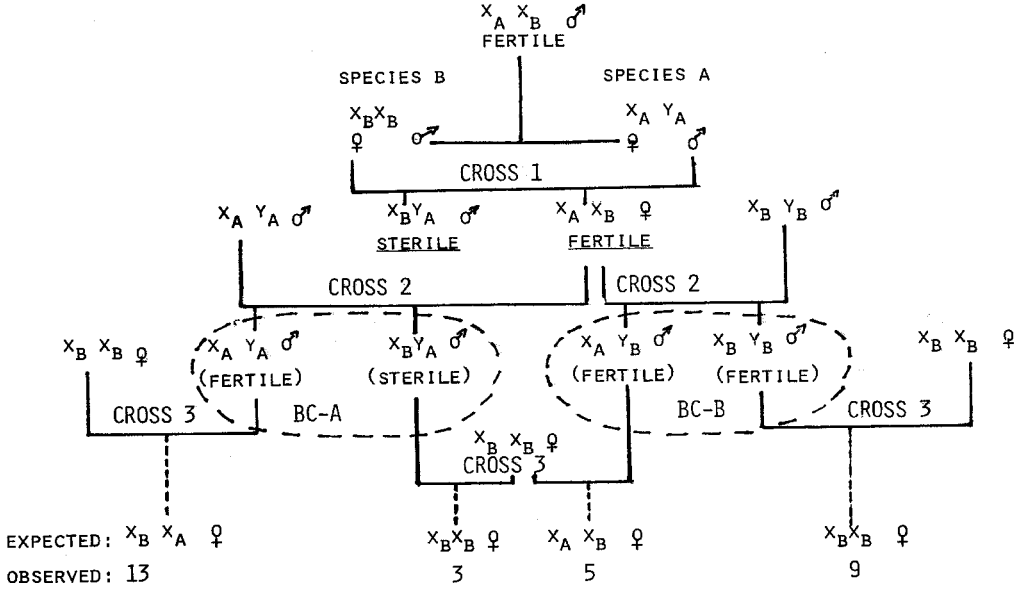

Fig. 1. Results of crossing *An. dirus* species B ♀ X *An. dirus* species A ♂ reciprocal crosses and male progeny of both backcrosses of the F_1 females. BC-A is backcross A and BC-B is backcross B. The F_1 males from the two reciprocal crosses were observed to differ in fertility and they are labelled sterile and fertile. On the hypothesis that the sterility is due to an adverse interaction of X_B with Y_A, the products of the backcrosses can be expected on this hypothesis are shown in parentheses.

Table 1. results of first backcrosses between X_AX_B females and Y_AY_A, X_BY_B males.

Backcross	Parental genotype	Number of: Crosses	Number of: Inseminations	Percent hatch	Number of males with spermatozoa in testes (number dissected)
BC-A	X_AX_B ♀ × X_AY_A ♂	69	24	70.7	57 (138)
BC-B	X_AX_B ♀ × X_BY_B ♂	75	20	64.5	43 (150)

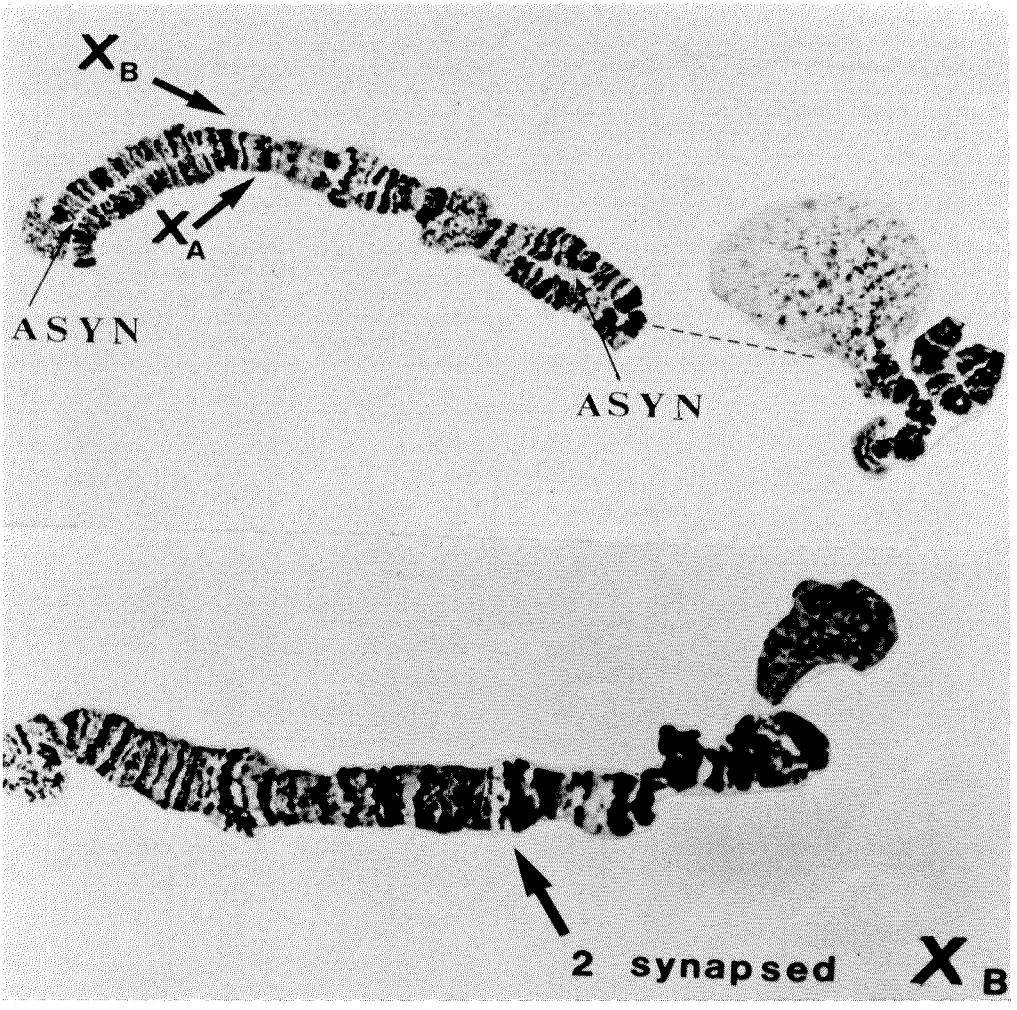

Fig. 2. Polytene chromosomes from larval salivary glands of backcross progeny:
A. X_AX_B female derived from backcrosses BC-A and BC-B as shown in Fig. 1. Note two largely asynaptic (ASYN) chromosomes one derived from each species.
B. X_BX_B female derived from the same backcross shown in Fig. 1. Note synapsed *An. dirus* species B X chromosome.

only 3 families that showed the typical synapsed X_B chromosome. These numbers differ significantly from a 1:1 ratio ($X^2_1 = 5.06$, $0.01 < p < 0.05$). In backcross BC-B there were 5 families that were heterozygous for the X chromosome (X_AX_B) and 9 families that were homozygotes (X_BX_B). These numbers do not differ significantly from a 1:1 ratio ($X^2_1 = 0.64$, $p > 0.05$).

DISCUSSION

On the hypothesis that the hybrid male sterility was due only to an interaction of the X and Y chromosomes, only the X_BY_A combination would be sterile because only the cross of *dirus* B females x *dirus* A males (and not the reciprocal) yielded sterile F_1 males. Thus only backcross BC-A (and not BC-B) would be expected to yield a proportion of sterile males. Furthermore, it would follow that all the fertile males from BC-A would be of the X_AY_A type and hence would yield X_BX_A female progeny when test-crossed to X_BX_B females. All the X_BY_A males among the BC-A progeny would, on the above hypothesis be sterile and thus no X_BX_B females would be found among the test cross progeny deriving from BC-A. These predictions were not fulfilled—there were many sterile males among the BC-B progeny and the proportions of sterile males was slightly higher than for BC-B than BC-A. Also X_BX_B females were produced by three of the test crosses of BC-A progeny. These observations certainly rule out an X-Y interaction as the whole explanation of the male sterility. However, there were significantly more X_AY_A than X_BY_A males among the fertile segregates from the BC-A backcross. This suggests that the X chromosome also plays a contributing role in causing hybrid male sterility. This role might be an interaction with the Y chromosome or autosomal factor or factors from the other species. The apparent absence of a major role for the X chromosome differs from the observations of Curtis (1978), Curtis et al. (1980) on the *An. gambiae* complex. Fraccaro et al. (1977) found a 1:1 ratio of sterile and fertile males in backcross progeny and concluded that an X-Y chromosome interaction was responsible for this segregation, but a single autosomal locus causing sterility when heterozygous could also explain their results.

It is possible that one or more of the following factors may explain the results from the crosses and backcrosses. They could be: (a) crossing-over between the X chromosomes in the females so that the cytologically identifiable X of species A or B origin does not always remain associated with a postulated male fertility factor(s) on the X chromosome. It is not known yet whether crossing-over can occur between the X chromosomes of the two *dirus* species in hybrid females; it does not occur between the X chromosomes of different *An. gambiae* sibling species (Curtis and Chalkley 1979); (b) an autonomous cytoplasmic factor which is inherited maternally regardless of the chromosome genome (C.F. Curtis, personal communication); (c) a cytoplasmic factor initiated by the maternal chromosomal gene; (d) the Y chromosome (Curtis et al. 1980). There is no direct evidence of the nature of the factor(s) apparently transmitted from the heterozygous females in the cross, but it seems probable that it would come in the category (a) and (c). Until a range of genetic markers are available further analysis of which chromosomes are involved in causing the sterility will not be possible.

The possibility of an X-autosomal interaction contributing to the sterility mechanism cannot be excluded since Mason (1964) shows that there are some autosomal effects on sterility in the *An. gambiae* complex. Davidson and Jackson (1962) concluded that their data fit almost exactly the proportions expected where a single, autosomal sex-limited (expressed only in the male) factor is involved, but they would also fit an X-Y interaction hypothesis as in the data of Fraccaro et al. (1977). Further studies are required to determine if sterility of this nature is linked with hybrid vigor and if there are any possible associations with mating ability and mate recognition systems. Understanding of the genetic and cytogenetic basis of sterility may enable one to determine the exact mechanisms of hybrid sterility and hopefully its potential for genetic control.

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