Isolation of a Homozygous X-linked Translocation Stock with two Additional Sex-Chromosomes in the Onion Fly *Hylemya antiqua* Meigen

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**Summary.** The onion fly, *Hylemya antiqua* Meigen, was subjected to irradiation and selection based on observations of fertility and cytogenetics, in order to isolate structural chromosome mutations which might be used for genetic control of this species. To the present time, only a "simple" X-linked translocation could be obtained as a homozygous stock. Sibcrossing was carried out using translocation trisomics (TN + X) obtained from testcrossed translocation heterozygous females (TN) showing numerical nondisjunction. A homozygous stock was obtained with two additional sex-chromosomes. This is a unique case because normally an X-linked translocation cannot be made homozygous in the male sex, which normally only carries one X-chromosome.

**Introduction**

Mankind is continually confronted with noxious insects, causing damage to agricultural crops and products, or functioning as vectors of diseases, especially in the tropics. Because of the adverse side-effects of chemicals, attempts are being made to develop biological (including genetical) was of controlling insect pests, by methods which cause less environmental pollution and which avoid the problem of insecticide resistance (Woods 1975). The onion fly, *Hylemya antiqua* Meigen, an important pest insect, was chosen in the Netherlands in 1965 as the "target" for a genetic control project. The sterile insect release method was given the main emphasis in the first few years and recently field releases of fully sterilized insects on a small scale (1 ha) appeared to be successful (Loosjes 1974).

Since 1969 the induction, isolation and cytogenetic analysis of structural chromosome mutations in the onion fly has become part of the genetic control project (van Heemert 1975). The use of chromosomal translocations (and inversions (Robinson and van Heemert 1975, Robinson 1975)) has been considered in several other insect pests (Baker and Sakai 1974, Curtis 1971, Foster and Whitten 1974, van Heemert 1975, Laven et al. 1971, Lorimer et al. 1972, McDonald and Overland 1973a, Rai et al. 1974, Wijnands-Ståb and van Heemert 1974, van Zon and Overmeer 1972). Such chromosomal rearrangements could be applied in two different ways. Firstly, "semi-sterility", that is associated with translocation heterozygotes, could be used to depress the population fertility. "Semi-sterility" is caused by duplication-deficiency gametes originating from adjacent I or II orientations of the translocation multivalent during meiosis. Following fusion of such duplication-deficiency gametes with normal gametes, unbalanced karyotypes occur which usually die during the embryonic stage, thus decreasing egg hatch (van Heemert 1973). This partial sterility is conventionally referred to as "semi-sterility" but, in fact, most of the translocation stocks which we have isolated, had sterilities greater or less than 50%. The combination of different translocations can give much higher sterilities. Secondly, a translocation could be used as a genetic transporting mechanism. By linking a deleterious gene close to one of the translocation breakpoints, a population could be completely replaced by the modified population after release of a sufficient majority of this type. Both methods require the production of homozygous translocation strains, to reproduce the translocation in large numbers without the occurrence of "semi-sterility", and to produce heterozygotes in any desired proportion of the population to be controlled. However several authors have found that a certain degree of sterility still occurs in most homozygous translocation stocks probably due to inbreeding, position effects and deleterious genes linked to the translocation (LaChance et al. 1964, Lorimer et al. 1972, McDo-
The translocation used in this study was induced by irradiating young adult males with 1.0 krad of X-rays (250/25 deep therapy apparatus, operating at 250 kVp and 15 mA, with a dose rate of 200 rad/min) (Wijnands-Stäb and van Heemert 1974). It was selected after backcrossing females and screening for "sterility". The chromosomes involved in the translocation can be recognized easily at several developmental stages (eggs, larvae and male adults). A normal karyotype of the onion fly consists of 10 submeta-centric autosomes and two small acrocentric sex-chromosomes (Fig. 1A-D). In the translocation, about half of the long arm of chromosome 3 is attached to the most distal segment of the X-chromosome. Apparently none, or only a very thin piece, of the small sex-chromosome is translocated to chromosome 3 and only chain quadrivalents were observed in male meiotic prophase (Fig. 2E). From earlier studies we concluded that it is most probably a simple (i.e. nonreciprocal) translocation (van Heemert 1974a). It was proved that the sex-chromosome involved in the translocation is the X-chromosome because, in testcrosses of translocation heterozygous males, only normal sons and translocation heterozygous daughters occur, whereas heterozygous females produce both normal and translocation sons and daughters. The karyotype of TN (+) may therefore be symbolized 33XXY. Figure 1A shows diagrammatically how this translocation originated after irradiation. Further investigations showed the presence of translocation trisomics (TN + X) which possess one additional X-chromosome compared to translocation heterozygotes (TN) (van Heemert 1974b). Figure 1B shows how numerical nondisjunction in TN (33XX) females can give rise to two unbalanced gametes. One carries an additional X-chromosome 33XXY, the other only has one normal chromosome 3 and no sex-chromosome. Fusion of a 3X gamete from the father with a 33XXY gamete will give a (33X3XY) = TN + X zygote (Fig. 1C). In females numerical non-disjunction can be observed regularly (19%), while in males it seldom occurs (2%) (van Heemert 1974a).

The flies were reared at 23°C, appr. 70% r.h. and 16 hours of light per day. As the onion fly hardly mates in single pairs (appr. 10% success) we mass-mated the flies and separated individual females in small cages (females only mate once) when oviposition had started. Eggs were incubated for three days at 29°C and nearly 100% r.h. in an oven. To measure sterility, eggs were classified as white (unfertilized), empty (hatched larvae) and brown (late embryonic lethals), using a stereomicroscope (12×). The sterility was defined as the percentage of brown eggs after the exclusion of white eggs from the total. As shown by van Heemert (1975) white eggs are unfertilized and their frequency is very low (3%) in crosses involving translocated and non-translocated flies. The percentage of brown eggs in the control is approximately 3%. Both testcrossed TN and TN + X males and females had an average sterility of about 25–35%. Cytological observations were made on eggs (8–16 hours), larvae (5–10 days), young males (1 day) and sometimes females (1 day). No special fixation of the tissues was needed after dissecting these tissues in Levy's saline solution. Larval brains were put in lactic acetic orcein directly after dissection. Testes and ovaries were first put in a hypotonic solution for 5 or 10 minutes, respectively, to obtain a swollen tissue before staining. Squashing was carried out after half an hour in 45% acetic acid. Cytological analysis was usually carried out immediately. Photographs (Figs. 2A-L) were made from temporary preparations with a Zeiss photomicroscope using a high contrast Agfa-Gevaert ortho negative film (12 DIN) or an 11 ford pan film (18 DIN).