TRANSLOCATIONS AND SEX RATIO DISTORTION IN THE ONION FLY, *HYLEMYA ANTIQUA* (MEIGEN), AND THEIR RELEVANCE TO GENETIC CONTROL.

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 24 oktober 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

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STELLINGEN

1. De zeer lage frekwenties, c.q. afwezigheid van adjacent 2 segregatie in mannelijke translocatieheterozygoten van de uievlieg vinden hun uiteindelijke oorzaak in de wijze waarop de meiotische chromosoomassociatie in deze sexe plaatsvindt.
   Dit proefschrift.

2. De door Keith Reid en Wehrhahn voor de uievlieg aanbevolen methode om translocatiehomozygoten te isoleren, is vanuit een praktisch oogpunt gezien inefficiënt en bovendien wordt in deze methode onvoldoende rekening gehouden met een homozygoteringsdepressie als gevolg van inteelt.
   Keith Reid, J.A. en C.F. Wehrhahn(1976). Canad. Entomol. 108,1409-1415.
   Keith Reid, J.A. en F.L. McEwen(1977). Canad. Entomol. 109,1287-1291.

3. Een genetische bestrijding met behulp van de zogenaamde "populatievervangingsmethode" biedt weinig perspectieven wanneer gebruik gemaakt wordt van conditionele lethaliteit.

4. Een numerieke variatie voor chromosoom Y_2 tussen primordiale, germinale cellen is hoogstwaarschijnlijk de belangrijkste oorzaak van het voorkomen van significant van de 1:1 verhouding afwijkende sex-ratio's in nakomelingschappen van XXY_2 mannetjes van de uievlieg.
   Dit proefschrift.

5. Een afzonderlijke klassificatie van alternate 1 en 2 oriëntaties (Endrizzi, 1974; Cochran, 1976 en 1977; Lacadena en Candela, 1977) is niet zinvol wanneer het doel is nadere informatie over de factoren die van invloed zijn op de oriëntatie van translocatiequadrivalenten te verkrijgen.
   Endrizzi, J.E.(1974). Genetics 77, 55-60.
   Cochran, D.G.(1976). Chromosoma 59, 129-135.
   Cochran, D.G.(1977). Chromosoma 62, 191-198.
   Lacadena, J.R. en M. Candela(1977). Chromosoma 64, 175-189.

6. In de vaktijdschriften voor land- en tuinbouw wordt onvoldoende aandacht besteed aan de nevenwerkingen van bestrijdingsmiddelen.

7. Onderzoek naar biologische, inclusief genetische, bestrijdingsmethoden van het aardappelcystenaaltje, *Globodera rostochiensis* en *G. pallida* , zal naast de resistentieveredeling een hoge prioriteit moeten verkrijgen.

8. Accijnsheffingen op bestrijdingsmiddelen kunnen een doeltreffende maatregel zijn om een doelmatiger gebruik van deze middelen te bevorderen; de revenuen zullen echter weer ten goede aan de land- en tuinbouw moeten komen.

9. Het zelfstandig leren opzetten en uitvoeren van eenvoudige onderzoeksexperimenten zou een essentieel element van het hoger agrarisch beroepsonderwijs moeten zijn; de faciliteiten hiervoor zijn echter onvoldoende.

10. Zowel biologisch als sociaal gezien is het logisch dat bij gelijke rechten van vrouw en man, het kind de familienaam van de moeder krijgt.
    Forel, A. Het sexueele vraagstuk. Amsterdam: Gebr. Graauw 1907. (Nederlandsche bewerking R. A. Oosterhout).

11. Om althans de jager het gevoel te kunnen blijven geven dat hij op wild jaagt, zal bij de kweek van voor de jacht bestemde fazanten een negatieve selectie op "wilde" eigenschappen zoveel mogelijk vermeden moeten worden.

L. Vosselman
Translocations and sex ratio distortion in the onion fly, *Hylemya antiqua* (Meigen), and their relevance to genetic control.
24 oktober 1980

VOORWOORD

In de eerste plaats mijn excuses aan alle medewerkers van de vakgroep Erfelijkheidsleer die de afgelopen jaren door mijn "bio-industriële" activiteiten voortdurend zijn geconfronteerd met de meest onwelriekende geuren. De geneeskrachtige werking die toegedicht is aan de geur van de ajuin, moge een troost zijn voor degenen die geteisterd werden door de dampen afkomstig van meer dan 2 ton uien.

Mijn grote dank gaat uit naar mijn promotor Prof. J. Sybenga voor de begeleiding van dit onderzoek. Ik heb zeer veel waardering voor het feit dat ondanks de zeer drukke werkzaamheden er altijd ruimschoots tijd beschikbaar was voor overleg en dat het corrigeren van de manuscripten op een zeer zorgvuldige en constructieve wijze is geschied.

Prof. J.H. van der Veen ben ik zeer erkentelijk voor de grote gastvrijheid en de ruime bijdrage aan de goede en plezierige werksfeer op de vakgroep Erfelijkheidsleer. Deze goede werksfeer is voor mij een belangrijke stimulans bij het onderzoek geweest. De directie van het ITAL zou ik willen bedanken dat zij mij financieel in staat gesteld heeft het praktische gedeelte van dit onderzoek te kunnen voltooien.

Kees van Hemert heeft door zijn pionierswerk, vooral wat betreft de cytologie van de uievlieg, ervoor gezorgd dat ik geen onbetreden paden behoefde te bewandelen. Als student heb ik onder jouw hoede kennis gemaakt met de uievlieg en het heeft me verheugd dat ik in een later stadium jouw plaats op Erfelijkheidsleer mocht innemen. Onze samenwerking heeft ertoe geleid dat twee artikelen van dit proefschrift onzer beider namen dragen. Alan Robinson zou ik willen bedanken voor zijn kritische kanttekeningen en de vele correcties van het Engels in de manuscripten. Bovendien ben jij de hoofdauteur geweest van het laatste artikel in dit proefschrift. Door jouw constructieve ideeën en niet aflatend optimisme was het een groot genoegen om met jou samen te werken. Wim van de Brink en Gijs Schelling worden bedankt dat zij bereid waren af en toe een deel van de kweek over te nemen. De leden van de IPO-uievliegwerkgroep van weleer zou ik willen bedanken voor de goede relaties en de faciliteiten waarvan ik gebruik mocht maken.

Henk Nijhoff, onze overeenkomsten in maatschappelijke en ruimtelijke positie op het lab, zijn waarschijnlijk goede condities geweest voor het feit dat wij uitstekend als communicerende vaten bleken te functioneren. Met meestal een bekertje koffie als spraakwater hebben we heel wat hypothesen en ideeën omtrent muizen en vliegen uitgewisseld; de direkte omgeving zal het niet ontgaan zijn dat ook "koetjes en kalfjes" geliefd waren. Ik zal altijd met veel genoegen hieraan terugdenken.

Het vele typewerk dat voor de totstandkoming van dit proefschrift nodig was, is voor het merendeel uitgevoerd door Henriët Boelema en in latere fasen heeft ook Trees Makkes een belangrijke bijdrage geleverd; voor de concentieuze en snelle wijze waarmee dit gebeurd is, ben ik jullie zeer erkentelijk. De heer Knoop en Hans de Vries zou ik willen bedanken voor het afdrukken van de diverse foto's en het maken van de collages. Jan Maassen heeft op een voortreffelijke wijze een groot deel van de illustraties verzorgd. Theo van Lent heeft het mogelijk gemaakt dat de vliegen en larven op een efficiente wijze opgekweekt konden worden. Jan Molenveld wordt bedankt voor een tijdelijke ondersteuning van de kweekwerkzaamheden. De overige medewerkers van de vakgroep Erfelijkheidsleer zou ik willen bedanken voor ofwel hun bijdrage aan dit onderzoek ofwel de plezierige kontakten die ik met hen gehad heb; de vele (ont)spannende ping-pong partijtjes zal ik niet snel vergeten.

Piet Dijkstra, jij, als welbekende uientapper, hebt gedurende een bepaalde periode aan den lijve kunnen ondervinden dat het tappen van een ui en het snijden van een siepel meestal tot hetzelfde eindresultaat leidt, namelijk siep(el)ogen. Ook Ep Mul heeft dit kunnen ervaren. Mijn dank, dat jullie een deel van je vrije tijd hebt willen opofferen.

Marijke Neijzing en Arie Bruinink hebben tijdens hun doctorale fase gemerkt dat de uievlieg een lastig proefkonijn is wanneer het gaat om banderen van chromosomen, respectievelijk kweken van larven en isoleren van DNA. Marion Herfst heeft kunnen constateren dat de uievlieg wel van een winterslaapje houdt. Mijn dank voor jullie bijdrage aan het onderzoek.

Mijn ouders ben ik dankbaar dat zij mij de gelegenheid hebben gegeven om te gaan studeren en dat ik ook af en toe praktizerend boer mocht zijn. De door jullie geteelde uien bleken goed voedsel voor de larven te zijn.

Elly, jij hebt de afgelopen jaren moeten ervaren dat zelfs vliegen voor je partner interessante objecten zijn. Aan de soms vurige wens van je, dat ze beter allemaal "de lucht in konden vliegen" heb je gelukkig geen gestalte gegeven, Integendeel, jij hebt diverse malen actief meegeholpen en het is een gelukkige constatering dat uiensoep nog steeds een lievelingsgerecht van je is.

CONTENTS

GENERAL INTRODUCTION

There is an increasing demand for the development of pest management systems in which the use of pesticides is reduced to a minimum. A prerequisite is that the alternative control methods are effective and are also economically attractive. Involved in such system are methods based on the introduction of specific alterations in the genetic make up of insect populations leading to reduced reproduction rates. This thesis will treat some aspects of the feasibility of using translocations and sex ratio distortion for genetic control of the onion fly, *Hylemya antiqua* or *Delia antiqua*.

In Western-Europe the onion fly is the only important insect pest of the onion and in the absence of control measures larvae of this fly can cause high damage levels and therefore insecticides are generally applied. Until 1965 organochlorine compounds (DDT, aldrin, dieldrin and heptachlor) were mainly used but subsequently they were replaced by organophosphorous compounds or carbamates. In the last ten years the organophosphorous compound trichloronate has been almost exclusively applied (Loosjes, 1976).

The awareness of the harmful side-effects and the accumulation in food chains of the broad-spectrum and very persistant organochloric compounds used in the fifties and sixties together with the occurrence of resistance against these insecticides, were the main reasons that some fifteen years ago in the Netherlands, research was initiated to develop genetic control methods for the onion fly. Without doubt the successful control of the screwworm in the U.S.A. with the "Sterile Insect Release Method"(SIRM) was an important stimulus to start this project. Following detailed studies on different aspects of the SIRM, a research team of the Institute of Phytopathological Research (IPO) has succeeded in applying this method for the onion fly. Recently in large-scale field experiments (about 40 hectares) it was demonstrated that an effective control of the onion fly with the release of sterilized flies is possible.

In 1969 at the Institute for Atomic Sciences in Agriculture (ITAL) and the Department of Genetics of the Agricultural University studies were started to establish the feasibility of using structural chromosome mutations for genetic control of the onion fly. In the main, reciprocal translocations were investigated. Translocations might be useful for insect control because the heterozygotes (T+) normally show a reduced fertility caused by the production of unbalanced (duplication/deficiency) gametes. In general,

heterozygotes carrying one reciprocal translocation produce about equal numbers of balanced and unbalanced gametes. However, in multiple translocation heterozygotes higher frequencies of unbalanced gametes are formed, resulting in lower fertilities. Translocation homozygotes, if viable, are expected to show normal fertility and are therefore especially suitable for mass-rearing.

When homozygotes are released into a natural population, a mixed population of ++,T+ and TT types will arise in subsequent generations. Since, however, heterozygotes are at a disadvantage due to their reduced fertility an unstable equilibrium will normally be present. Consequently, a gradual shift in karyotype frequencies and in sterility level will occur until finally fixation of either ++ or TT takes place. To preclude such a fixation a monotoring of the population and additional releases of TT (or ++) after a certain number of generations would be necessary. Nevertheless, a single release of TT-individuals can result in a higher total genetic load compared to a single release of an equal number of completely sterile individuals. This is because fully sterile individuals have no effect upon the population fertility in subsequent generations. It should be realized, however, that the reduction in population fertility following the release of a single translocation is mostly too low for the control of an insect. An enhancement of the sterility level is possible if more translocation strains are released but then it will be more difficult to obtain and to maintain an "optimal" ratio of the different karyotypes.

Translocations can be exploited in several ways for insect control. In this thesis attention will be mainly paid to fertility reduction of populations based on the release of translocation homozygotes (see above). A second potential use of translocations in insect control are multiple translocation heterozygotes showing high levels of sterility. These can be produced by intercrossing of (double-)homozygotes. Such highly sterile heterozygotes might be substituted for radiation sterilized individuals (in the SIRM), since a sterilization by means of irradiation often results in a reduced competitiveness. Thirdly, translocations can be used as "vectors" of genes causing conditional lethality or preventing disease transmission (refractory genes). Since no experimental work has been done on this subject, this method will only briefly be discussed.

Sex ratio distortion in favour of males can be of great importance for the genetic control of insects if for instance 1) only females cause damage

(Mediterranean fruit fly), 2) only females transmit diseases (several mosquito species), or 3) for a sterilization of females a higher dose of irradiation is needed than for males (a higher dose can have an adverse effect on the competitiveness). In *H.antiqua* it is for economic reasons that sex ratio distortion in favour of males is important. Since males of *H. antiqua* are polygamous it is expected that in the SIRM only males cause a significant contribution to the reduction of the population fertility. Therefore, if with an appropriate crossing scheme only or predominantly males could be obtained, then about half of the mass rearing costs would be required. For genetic control based on translocations, sex ratio distortion might be of even greater importance. When fully fertile translocation homozygotes are released to achieve an equal ratio of normal (+) and translocation (T) genomes in a target population it is necessary to double the reproducing population. By releasing only translocation homozygous males such as doubling of the reproducing population can be avoided. For these reasons detailed attention has been paid to the genetics of a sex ratio distortion mechanism in *H. antiqua* which was discovered during the research.

The results of this sex ratio distortion mechanism are given in the first two chapters of this thesis. A selective killing of females with the aid of a Y-autosome translocation and a locus causing conditional lethality (located on the translocated autosome) gives another opportunity to manipulate sex ratios. This theme comes up for discussion in the chapters 3 and 4. The main subject of the chapters 3, 4 and 5 is the meiotic segegration behaviour of translocations in relation to fertility. Different types of translocations were studied: a highly fertile Y-linked translocation (chapter 3), a Y-linked three-chromosome double-translocation (chapter 4) and five different autosomal reciprocal translocations (chapter 5). Embryonic and larval mortality percentages have been compared with the frequencies of duplication/deficiency karyotypes observed in eggs of testcrosses and/or MII cells of males. Intrinsic factors of the multivalents which most probably affect the mode of segegration and therefore the frequency of duplication/deficiency karyotypes are discussed. Due to larval rearing difficulties it was only possible to test five translocations, including three double-translocations, in respect of the viability of the homozygotes. For one translocation a pure breeding stock of homozygotes was obtained. The isolation procedure used is discussed in chapter 6.

Competition experiments with these homozygotes were carried out in laboratory (chapter 6) and field (chapter 7) cages. Finally, the egg-hatch and pupation percentages of some double-translocation heterozygotes were determined. The results of these studies have not been included but in the general discussion a short summary is given together with data on meiotic segregation of two of these double-translocation heterozygotes.

Chromosoma (Berl.) 67, 201–218 (1978)

Sex Determination of the Onion Fly, *Hylemya antiqua* (Meigen)

I. Sex Chromosome Polymorphism, Gynandromorphism and X-Polysomy

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Abstract. X and Y chromosomes of Hylemya antiqua occur in two forms each. X_L and X_S, and Y_1 and Y_2. The larger X_L has an intercalary proximal segment which is absent in the more common smaller X_S. The acrocentric Y chromosome (Y_1), does not differ morphologically from X_S. A smaller metacentric Y_2 is apparently not homologous with Y_1. Two types of males, XY_1 and XXY_2, coexist in at least one Dutch population. XY_2 has been observed in one individual only. In larval ganglion cells an association has been observed between chromosome Y_2 and a probably non-homologous, intercalary segment of autosome 4. A numerical somatic variation of Y_2 can lead to gynandromorphs and sex ratios significantly different from 1:1. XX cells can differentiate into functional spermatozoa in XX/XXY_2 mosaic testes. This indicates the presence of a diffusable male determining substance, which can reverse the "genotypic" sex of a cell. The occurrence of some spermatozoa-containing "cysts" in ovaries of two gynanders suggests a more or less autonomous (independent of the gonadal environment) differentiation of XXY_2 germ cells. $XXXY_2$ males and XXX females do not show a serious reduction in fertility. Even $XXXXY_2$ males do not exhibit any sign of intersexuality and spermatogenesis seems unaffected. All 62 scored M II cells of X-tetrasomic males contained 2 Xs.

Introduction

Sex determination in Diptera is based on several different systems. Using aneuploid individuals, expecially polysomics for X, the existence of (a) strongly male determining factor(s) on the Y chromosome in Dipteran species has been established e.g. in *Phormia regina, Lucilia cuprina* and *Chrysomya chloropyga* (Ullerich, 1963, 1976), *Pales ferruginea* (Ullerich et al., 1964), *Musca domestica* (Hiroyoshi, 1964; Milani et al., 1967) and *Hylemya antiqua* (van Heemert, 1974b). Beside this so called "dominant Y" system, several other sex determination mechanisms are known in the Diptera (see White, 1973).

Using an X-linked translocation, van Heemert (1974a, b) could confirm Boyes' (1954) suggestion that the small acrocentric chromosome pair in the onion fly (*H. antiqua*) is the sex chromosome pair. According to van Heemert (1974a, b) the difference between the X and Y should be the presence and absence resp. of a quite proximal secondary constriction. Boyes mentioned a slight difference in length and absence/presence of a submedian secondary constriction as possible differences between X and Y.

Onion flies with some intermediate secondary sex characters but with normal testes are observed rather often (a few percent) in laboratory rearings, but in wild populations only three of 7057 individuals were of this type (Loosjes, 1976). An "androgynous" onion fly was reported by Tiensuu (1935). In other *Hylemya* species comparable aberrations in sex phenotype have been mentioned (Hennig, 1974; Sick, 1967; Smith, 1972).

Following the careful analysis of the sex determining system in *H. antiqua* by van Heemert (1974a, b) gynandromorphs and very deviant sex ratios were observed which could not be explained by his theory of an XX/XY sex determination. In addition, it became probable that a very small metacentric accessory chromosome, formerly considered a B chromosome, could play a role in sex determination. It does not pair somatically or meiotically with the sex chromosomes. These observations, together with the notion that sex ratio distortion may be a useful tool in genetic control of insects, prompted the present analysis. Further aspects will be presented elsewhere (Vosselman, in prep.).

Materials and Methods

The wild stock used was originally collected in Zeeland (southwestern part of the Netherlands) and reared for five generations in high numbers under laboratory conditions at the Institute for Phytopathological Research, Wageningen. Stock T14 with a reciprocal translocation between the autosomal chromosomes 2 and 6 was obtained from the Institute for Atomic Sciences in Agriculture (ITAL) in Wageningen. This stock has been backcrossed with the wild stock for more than three generations with always the wild type as male parents. X-polysomic flies have been derived from a trisomic arisen spontaneously in this stock. The translocation will be the subject of later papers.

The adult flies are normally reared at 23° C, a relative humidity of approx 70% and 16 h daylight. Cages made of clear perspex pipe of 7 cm diameter and 10 cm high have been used. The upper and lower sides are covered with nylon gauze. The cage is positioned on top of a small box with onion segments. Between cage and box a perforated piece of hard pvc (immediately below the cage) and a small circle of filter paper are placed. Attracted by the smell of onion, the females oviposit through the holes in the pvc and onto the filter paper.

The larvae are reared in plastic boxes of approximately 300 cm^3 on pieces of onion. The lower side of the box is perforated (an covered with fine nylon gauze) to prevent an excess of moisture in the box. In the box on a layer of vermiculite a pvc ring (diameter 8 cm) with coarse nylon gauze is placed. At the time of pupation the larvae move through the gauze into the vermiculite and pupate there; the remnants of onion can be taken out with the ring. Before the filterpaper with eggs is placed on the onion, the eggs and paper are desinfected with a 1.0% formaldehyde solution. For good larval growth and low mortality, the percentage of moisture in the box is very important; is it too high then a souring of the onion can take place, is it too low then there is a risk that the outside of the onion pieces becomes too hard for the larvae to penetrate. The larvae are first reared at 25° C in the dark during 3 d and afterwards at 20° C, 16 h daylight and a relative humidity of approx. 70%. The pupation percentages (number of hatched eggs/number of pupae, multiplied by 100) were 70–100%.

The percentage of fertilized females is normally very low with "single pair mating". Van Heemert (1974a) reports 10%. Two factors play an important role for the degree of success of single pair mating:1) rearing conditions of the larvae, 2) duration of storage of the pupae before use (in the refrigerator at 4° C, starting 2–4 d after pupation). The best results were obtained without storing. The results given in Table 1 come from single pair matings (with about 70% fertilized females). The remaining results are obtained from progenies of single females, from cages with about 10 females and 10 males, which had been together during 1–2 d at an age of about 10–14 d.

As fixing-staining medium a 2% lacto acetic orcein solution (van Heemert, 1974a) was used. For karyotyping larvae (brains) the best results were obtained with 5–9 d old larvae, which had fed 1 d on a fresh piece of onion. To obtain a high number of spermatogonial metaphases, the best stage for classifying the sex chromosomes, testes were dissected 1–2 d before eclosion of the pupae. For classifying dividing somatic cells of ovaries, the best results are obtained when the ovaries are isolated 1–2 d after eclosion of the pupae. Dissection was carried out in Levy's saline solution (van Heemert, 1974a) or directly in distilled water. Photographs were made with a Zeiss photomicroscope on an Agfa-Ortho 25 professional film (12 DIN).

Results

I. Sex Chromosome Polymorphism

Four different sex chromosomes are postulated for the onion fly (diagrammatically represented in Fig. 1). There are two non-homologous Y chromosomes. The phylogeny of these chromosomes is not certain and therefore the longer one is called Y_1 and the smaller one Y_2 (cf. White, 1973). The homologous but in respect of length different X chromosomes are designated as X_L (=long) and X_S (=short). The centromeres of the homologous chromosomes X_S, X_L and Y_1 are positioned at the end of the chromosomes, no short arm being observable. These are called acrocentric rather than telocentric (also by Boyes, 1954 and van Heemert, 1974a, b).

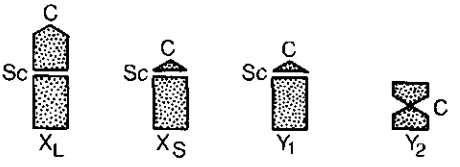

Fig. 1. Four different sex chromosomes of *H. antiqua*, diagrammatically. *C* centromere, *Sc* secondary constriction

Chromosomes X_S and Y_1

With the conventional staining techniques the X_S and Y_1 chromosomes can not be distinguished morphologically. Both are acrocentric and have a secondary constriction (SC) near the centromere (Fig. 2a). This constriction is not always visible, and within an individual (brains of larvae) one can observe cells where both, one or none of the acrocentrics have the SC. In respect of homology and morphological correspondence between X_S and Y_1 the following observations are relevant:

1) In several spermatogonial (pro)metaphases (Fig. 2a) the two acrocentrics

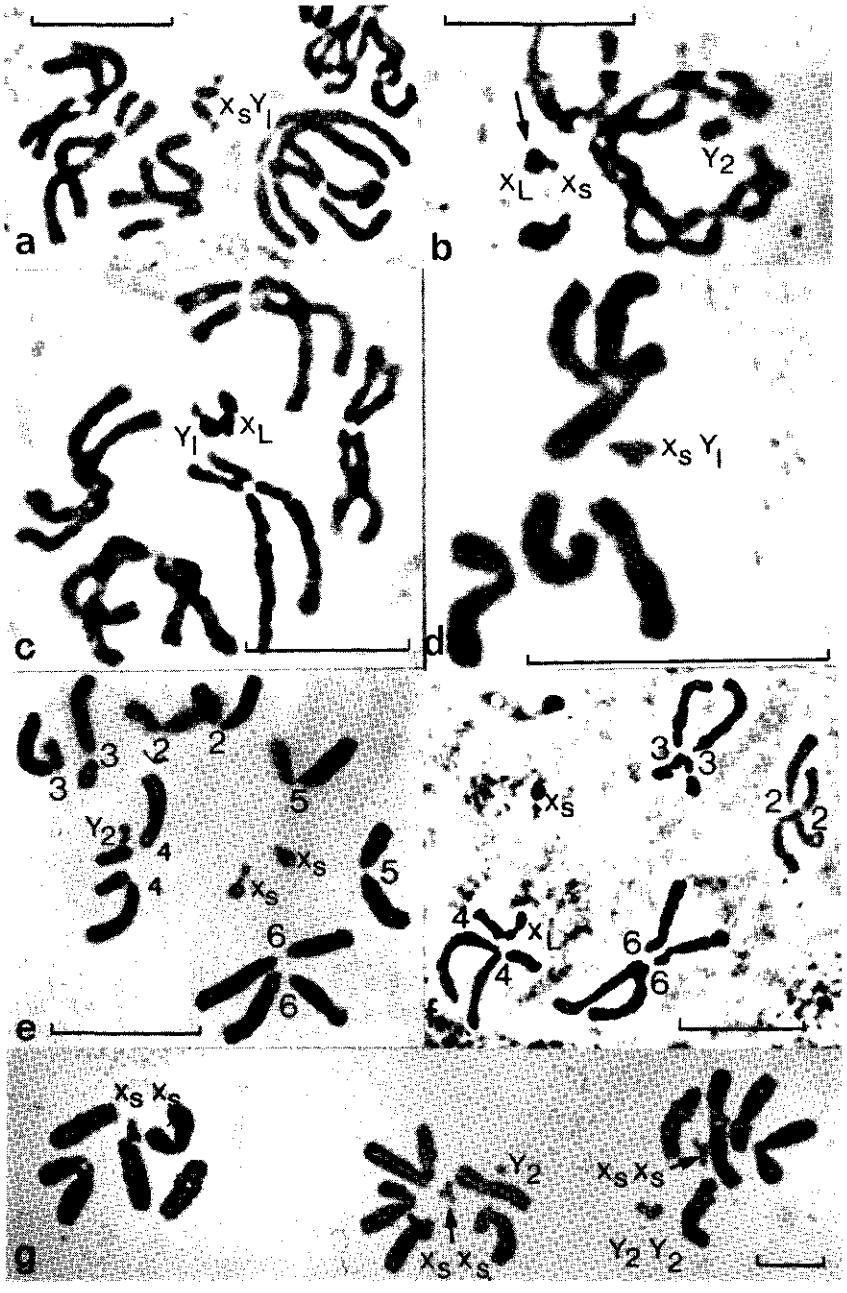

Fig. 2a–g. Karyotypes of *H. antiqua*. **a** $X_S Y_1$. Spermatogonial metaphase. X_S and Y_1 cannot be distinguished. **b** $X_L X_S Y_2$. Spermatogonial prometaphase, incomplete. Note difference in length of proximal segment. **c** $X_L Y_1$. Spermatogonial metaphase. **d** $X_S Y_1$. Diakinesis/prometaphase ♂. T+ for translocation T14. **e** $X_S X_S Y_2$. Mitotic metaphase larva. Note association of Y_2 with chromosome 4. **f** $X_L X_S$. Mitotic metaphase larva, incomplete. X_L associated with chromosome 4. **g** $X_S X_S Y_2$. Diakinesis/prometaphase ♂: $X_S X_S$, $X_S X_S Y_2$ and $X_S X_S Y_2 Y_2$ cell. The bars represent 10 μm

paired with each other for their whole length. In each of the two a SC was present.

2) In the progeny of the combination X_LX_L (♀) × X_LY_1 (♂) (see below) where the Y-chromosome is morphologically distinct from the X chromosomes, it was clearly observable that the Y_1 had the SC.

It is remarkable that the kinetochores and proximal chromosome segments of the acrocentrics disjoin in the male as early as diakinesis/prometaphase (Fig. 2d) when the homologues of the autosomal bivalents are still closely associated. This applies to X_SY_1 and X_SX_S bivalents. Both sex chromosomes X_S and Y_1 seem to be largely or perhaps even entirely euchromatic, because only very small dots of darkly stained chromatin can be recognised in interphase nuclei.

The frequencies of males and females in progenies of single pairs of the combination X_SX_S (♀) and X_SY_1 (♂) are given in the left part of Table 1. The ratio of females to males was in no case significantly different from 1:1. On the other hand in some of the crosses with $X_SX_SY_2(Y_2)$ as males, an excess either of females or of males is observed (see below). A reciprocal translocation between the autosomes 2 and 6 is present in this material, but it has no impact on the sex ratios.

Table 1. Frequencies of males and females in the progenies of single pairs of the onionfly. In the left part of the table (28d–51d) the crosses: X_SX_S (♀♀, stock a) and X_SY_1 (♂♂, stock b). In the right part the "reciprocal" ones: X_SX_S (♀♀, stock b) and $X_SX_SY_2$ (Y_2) (♂♂, stock a). Some males have a mosaic constitution for Y_2 (see text). A reciprocal autosomal translocation is present in the material used (see text). From the single pairs 33d, 36d, 38d and 42d a total of 20 adult males were cytologically screened, all were X_SY_1 (not shown in the table)

Single pair nr.	Number of ♀♀	Number of ♂♂	χ^2 (1:1)	Translocation karyotypes parents	Single pair nr.	Number of ♀♀	Number of ♂♂	χ^2 (1:1)	Translocation karyotypes parents	Karyotyped ♀♀ X_SX_S	Karyotyped ♂♂ $X_SX_SY_2(Y_2)$
28d	32	44	0.95	T+/T+	55d	41	53	1.53	++/++		
31d	87	80	0.29	++/++	56d	34	25	1.37	++/++		
32d	31	27	0.28	++/++	57d	7	86	67.11***	T+/T+	2	6
33d	53	73	3.17	T+/++	59d	68	37	9.15**	T+/++		
34d	27	31	0.28	T+/++	60d	52	46	0.37	T+/T+	4	7
35d	67	60	0.39	T+/++	62d	49	18	14.34***	++/++	2	5
36d	20	28	1.33	T+/++	63d	40	42	0.05	T+/++		
38d	43	43	0.00	T+/++	64d	35	37	0.06	++/++	2	10
39d	29	28	0.02	++/++	68d	35	28	0.78	T+/T+		
42d	59	64	0.20	++/++	69d	26	60	13.44***	++/++	4	4
49d	46	44	0.04	++/++	70d	50	50	0.00	++/++	3	3
51d	18	19	0.03	T+/++	73d	53	32	5.19*	++/++	4	3
					75d	33	65	10.45**	T+/++	3	6
					76d	37	44	0.61	++/++		
total	512	541			total	560	623			24	44

* $0.01 < p < 0.05$ ** $0.001 < p < 0.01$ *** $p < 0.001$

Table 2. Frequencies of karyotypes in the offspring of X_LX_S and X_LY_1 (♂). X_LX_L (♀) and X_LY_1 (♂). Chromosomes X_S and Y_1 cannot be distinguished morphologically. The individuals scored as larvae are only classified according to karyotype, the sex cannot be determined phenotypically in this stage

Crossing combination		Stage of scoring	Females		Males	
♀	♂		X_LX_L	X_LX_S	X_LY_1	X_SY_1
X_LX_S	X_LY_1	adults	7	5	4	5
X_LX_L	X_LY_1	larvae	5	–	8	–
X_LX_L	X_LY_1	adults	5	–	5	–

Chromosome X_L

In the progeny of certain crosses, individuals were observed with an acrocentric chromosome which is homologous for most of its length with X_S and Y_1 (Fig. 2b and c). But there is a clear deviation in length and in position of the SC. The distal part, beyond the SC seems to be completely homologous with the X_S and Y_1 chromosomes mentioned above. The proximal part, between SC and centromere, however, is much longer in the case of X_L, so that the place of the SC is submedian (Fig. 2c). There is no proximal SC. Despite the structural difference between X_L and X_S, Y_1 resp., there is a regular meiotic disjunction (AI). The additional chromosomal segment of X_L seems to be heteropycnotic, although we are not completely sure about this.

In progenies of the parental combination X_LX_S (♀) and X_LY_1 (♂) two distinct karyotypes were present in females as well as in males. In larvae only three karyotypes could be distinguished, because X_LX_S and X_LY_1 are morphologically identical. One offspring was found with exclusively X_LX_L females and X_LY_1 males (Table 2). No phenotypical differences in respect of the sexual characteristics between X_SX_S, X_LX_S and X_LX_L females, and between X_SY_1 and X_LY_1 males, were observed. There is no doubt that X_L behaves as a normal X chromosome.

Chromosome Y_2

A smaller and metacentric chromosome, here indicated as Y_2 and considered to be a B chromosome by van Heemert (1974a), differs from the other sex chromosomes in respect of a number of characters:

1) A numerical somatic variation is observable. 2) It is mostly present single. In meiosis it does not pair with the other sex chromosomes. In larvae there is a weak association with X_L, but a more pronounced association with chromosome 4.

ad 1. In brains of larvae and in testes of adults frequently a numerical variation being observable, i.e. within an individual there are cells with 0, 1, 2 or even more Y_2 chromosomes. More details about the degree of variation

will be represented elsewhere (Vosselman, in prep.). In Figure 2g an example of this variation is shown.

ad 2. Although Y_2 normally has no meiotic pairing partner, this has no direct implications for a regular course of meiosis. In anaphase I Y_2 goes undivided to one of the poles and in the second meiotic division a normal disjunction of the sister chromatids takes place. The possibility that occasionally nondisjunction of Y_2 in A II occurs, cannot be excluded (Vosselman, in prep.). If two or more Y_2 chromosomes were present, always a bivalent or multivalent is observed, never univalents.

In ganglion cells of larvae there is a clear association of Y_2 with chromosome 4, in the region between centromere and secondary constriction in the short arm of this chromosome (Fig. 2e). This association is not restricted to cells with only one Y_2. It is not as perfect as in average somatic pairing. Several cells can be found without this association, but probably "squashing effects" play a role. In testis preparations, in spermatogonial (pro)metaphases as well as in meiotic cells no association between Y_2 and chromosome 4 is observed. In larval ganglion cells also an association is sometimes found between the proximal part of X_L and the part of chromosome 4 (Fig. 2f) which associates with Y_2. This association, however, is much weaker and often not perceptible. It is not observed in testis preparations. In larvae with both X_L and Y_2 chromosomes a pseudomultivalent of all these chromosomes is sometimes seen.

The frequencies of males and females in progenies of single pair combinations X_SX_S (♀) and $X_SX_SY_2$ (♂) are shown in the right part of Table 1. Some ratios are significantly different from 1:1. In all males Y_2 was present. However, many had testes with a mosaic constitution for Y_2. The testes of most males consisted predominately of cells with 1 Y_2, but also males with a relatively high number of cells with 0 Y_2 or with predominately cells with 2 Y_2s were observed. The number $X_SX_SY_2Y_2$ karyotypes have not been presented here, because the frequency distribution of the average number of Y_2s per individual in the range 1.0 2.0 is not discontinuous (cf. Vosselman, in prep.)

The theoretical possibility that one of the acrocentrics might be a Y_1 chromosome (morphologically not different from X_S) and that the holandric inheritance of the metacentric chromosome was caused by a preferential disjunction in AI, i.e. that the metacentric would go to the same pole as Y_1, could not be excluded for such karyotypes. This problem could be solved by crossing males with the long acrocentric X_L. The frequencies of karyotypes found in the offspring of the parental combination X_SX_S (♀) and $X_LX_SY_2$ (♂) and of X_LX_L (♀) and $X_LX_SY_2$ (♂) are given in Table 3. There is no preferential segregation of Y_2 with regard to one of the acrocentrics and Y_2 is again only found in males. Apparently Y_2 is a strictly male determining chromosome. An $X_LX_LY_2$ karyotype is shown in Figs. 3a and 3b and a somatic metaphase of an X_LX_L female in Fig. 3c.

II. Gynandromorphism

The frequency of phenotypically clearly recognisable gynandromorphs is low. In Table 4 only those individuals from which also cytological information is

Table 3. Cytological data of the offspring of the parental combinations a) X_SX_S (♀) and $X_LX_SY_2$ (♂) b) X_LX_L (♀) and $X_LX_SY_2$ (♂). Some males and larvae had a mosaic constitution for Y_2 (see text). The larvae are classified according to the karyotype, the phenotypic sex is not known

Cross	Stage of scoring	Females			Males		
		X_LX_L	X_LX_S	X_SX_S	$X_LX_LY_2(Y_2)$	$X_LX_SY_2(Y_2)$	$X_SX_SY_2(Y_2)$
a	larvae	–	16	18	–	20	24
	adults	–	9	11	–	17	9
	total	–	25	29	–	37	33
b	larvae	3	3	–	1	5	–
	adults	5	6	–	4	6	–
	total	8	9	–	5	11	–

Table 4. Description of some morphological sexual characteristics and cytological data of 4 gynandromorphous types of the onion fly. 1) studied at pupal stage. s.met. = somatic metaphases

		gyn. 1	gyn. 2	gyn. 3[1)]	gyn. 4
External sexual characteristics	a) distance between eyes	about as ♂	about as ♂	–	as ♂
	b) position of wings	about as ♂	about as ♂	–	as ♀
	c) external ♂ genitalia ovipositor	intermediate form (see text)	intermediate form (see text)	♂-external genitalia (somewhat reduced)	normal ovipositor
Internal reproductive organs	a) ovary/ testis	ovary + testis, in ovary some spermatozoa present	ovary + testis, no spermatozoa in ovary	two ovaries; in ovary I appr. 150 spermatozoa	two ovaries
	b) number of spermatheca	two	two	none	three
Cytological results		ovary: s.met 5 cells: X_SX_S	ovary: s.met. 6 cells: X_SX_S	ovary I: s.met. 3 cells: X_SX_S 1 cell: $X_SX_SY_2$	ovaries: s.met. 19 cells: X_SX_S
		testis: dipl./ diak. 6 cells: $X_SX_SY_2$	testis: dipl./ diak. 2 cells: X_SX_S 3 cells: $X_SX_SY_2$	ovary II: s.met. 5 cells: X_SX_S	
			MII 3 cells: X_S		

present are given, and of these only the most striking characteristics. Two gynanders (1 and 2) had a female and a male gonad. The external genitalia were a mixture of male and female characteristics: an ovipositor-like organ, which could be pushed out, and partially developed male external genitalia at the top of this organ. Instead of the normal number of three spermatheca

(Theunissen, 1976) only two were present. Gynander 3, studied at pupal stage 1–2 d before eclosion, had normal but somewhat smaller male external genitalia. However, instead of testes two ovaries were present; no spermatheca could be detected. The ovaries had a normal appearance but a peculiarity was that a group of about 150 spermatozoa was present in one of the ovaries. In the female gonad of gynander 1 a comparable situation was observed with two groups of spermatozoa embedded in a cyst-like wall. It is hardly possible that the spermatozoa have come from the testis of this gynander, because the gonads were well separated before bringing them on different slides. Besides, the spermatozoa were embedded in "cysts". The ovaries in these gynanders did not seem different from normal ovaries, except for the presence of the spermatozoa and probably some more unidentifiable male germ cells. Several female germinal cell types could be recognised. Of gynander 4 it is not completely certain if it was a true gynandromorph; the only male characteristic was the relative closeness of the eyes. The other characters were all female.

The cytological data are not very extensive and in the case of female gonads only somatically dividing cells could be scored. It is not known from which part of the somatic tissue of the gonad these cells were derived. In the ovaries of gynanders 1 and 2 no Y_2 chromosome was observed, but in both testes $X_SX_SY_2$ cells were present, although one of the testes had a mosaic constitution (Table 4). In the spermatozoa-containing ovary of gynander 3 one of the four analysable somatic cells was clearly $X_SX_SY_2$.

One male without any external genitalia was observed; 24 cells (diplotene/diakinesis) could be karyotyped and all were X_SY_2 (Fig. 3d) except one, which was $X_SY_2Y_2$. This is the only case that Y_2 has been observed together with only one X chromosome. It is not likely that there is a causal relationship between these two facts, as will be discussed later.

III. X-Polysomy

The frequencies of karyotypes in the progeny of two X-trisomic parents (derived from a disomic/trisomic cross) are shown in Table 5. The karyotypes of the parents are in fact not known, but deduced from the (frequencies of) karyotypes in the offspring. Most probably the parents were $X_LX_LX_S$ (♀) and $X_LX_SX_SY_2$ (♂) or $X_LX_SX_S$ (♀) and $X_LX_LX_SY_2$ (♂). For a few individuals it was not possible to determine the exact karyotype (in respect of X_L/X_S) and for safety some karyotypes have been combined. The X-trisomic males (Fig. 3e) and females showed a normal viability and no serious reduction in fertility. For the X-tetrasomic males (Fig. 3h) no fertility scores are available, but no sign of intersexuality has been observed. Spermiogenesis seemed to be normal and an abundant number of sperm cells was present. X-tetrasomic females (Fig. 3f) were in any case viable.

The disjunction of the X-quadrivalent ($X_LX_LX_LX_S$ and $X_LX_LX_SX_S$) in four tetrasomic males was always very regular: 62 cells scored at M II all were 2 X. In diakinesis/M I (Fig. 3g) it was often seen that two centromeres of the quadrivalent are directed to one pole and the other two to the opposite

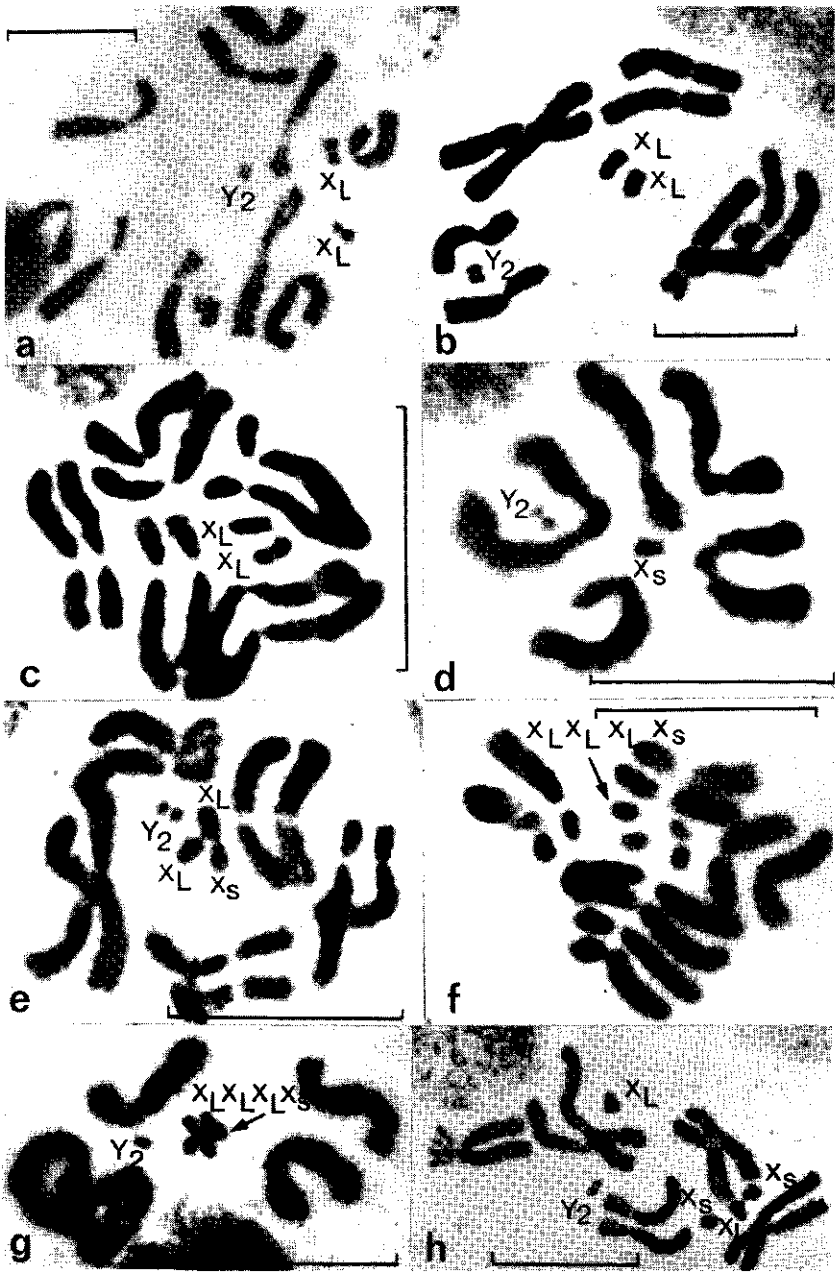

Fig. 3a–h. Karyotypes of *H. antiqua*. **a** $X_LX_LY_2$. Mitotic metaphase, larva. **b** $X_LX_LY_2$. Spermatogonial metaphase. **c** X_LX_L. Somatic metaphase in ovarium. **d** X_SY_2. Diakinesis/prometaphase ♂. **e** $X_LX_LX_SY_2$. Spermatogonial metaphase. **f** $X_LX_LX_LX_S$. Somatic metaphase in ovarium. **g** $X_LX_LX_LX_SY_2$. Diakinesis/prometaphase ♂. **h** $X_LX_LX_SX_SY_2$. Spermatogonial metaphase. The difference between X_L and X_S in **e, f, g** and **h** is not always clear, but the given karyotypes are based on several cells. The bars represent 10 μm

Table 5. Offspring of the cross $X_LX_LX_S$ (♀) and $X_LX_SX_SY_2$ (♂), or $X_LX_SX_S$ (♀) and $X_LX_LX_SY_2$ (♂): the parental karyotypes are not known, but deduced from the presence and relative frequencies of karyotypes in the offspring. In a few cases it was not possible to determine the exact karyotype, and therefore some have been combined

Karyotypes		Observed No		Total No (♀♀ + ♂♂)		
♀♀	♂♂	♀♀	♂♂	Obs.	Exp.[a]	Exp.[b]
X_LX_L	$X_LX_LY_2$	0	2	2	2.1	–
X_LX_S	$X_LX_SY_2$	1	4	5	5.3	9.5
X_SX_S	$X_SX_SY_2$	1	0	1	2.1	–
$X_LX_LX_L$	$X_LX_LX_LY_2$	0	0	0	1.1	–
$X_LX_LX_S$ $X_LX_SX_S$	$X_LX_LX_SY_2$ $X_LX_SX_SY_2$	4 + 2[c]	11 + 1[c]	18	17.0	19.0
$X_SX_SX_S$	$XX_SX_SY_2$	0	0	0	1.1	–
$X_LX_LX_LX_S$ $X_LX_LX_SX_S$	$X_LX_LX_LX_SY_2$ $X_LX_LX_SX_SY_2$	7	4	11	7.4	9.5
$X_LX_SX_SX_S$	$X_LX_SX_SX_SY_2$	0 + 1[c]	0	1	2.1	–

[a] Numbers expected on the basis of random coorientation of the different X chromosomes
[b] Numbers expected on the basis of coorientation exclusively between fully homologous X chromosomes
[c] Scored as larvae

pole (note: never 2 bivalents in the achiasmate males). In the right columns of Table 5 the expected numbers of karyotypes are given: in the case of 1) random coorientation of the different X-chromosomes in both parents 2) a coorientation exclusively between fully homologous X-chromosomes in both parents.

Discussion

Chromosomes X_S, X_L and Y_1

With an X-linked translocation van Heemert (1974a, b) established that the small acrocentrics are sex determining chromosomes. A morphological differentiation between the X and Y was suggested to be a secondary constriction in the X, which was found missing in the Y chromosome. Although van Heemert (personal communication) studied the chromosomes which are here indicated as X_S and Y_1, we have not been able to confirm the morphological difference between these chromosomes. In several spermatogonial prometaphases of different males two acrocentrics, pairing for the whole length and both with a secondary constriction have been observed (Fig. 2a). Also, the possible differences between X and Y which were suggested by Boyes (1954) seem not to hold true in our material.

In the same population where X_S and Y_1 were found, a few individuals were present with a longer acrocentric chromosome with one submedian secondary constriction. The results of hybridization experiments (Table 2) between these individuals and flies with the X_S chromosomes, clearly demonstrate that

this chromosome is an X chromosome (X_L). In the progeny of the cross $X_L X_S$ (♀) $\times X_L Y_1$ (♂) only females were homozygous for the longer acrocentric and only males had a homomorphic pair for the small acrocentric (X_S and Y_1 morphologically identical). However, a heteromorphic pair could be present in both males as well as females. Full-sib mating in the above-mentioned progeny has given an offspring with exclusively homomorphic ($X_L X_L$) females and heteromorphic ($X_L Y_1$) males.

It is not certain which chromosome (X_L or X_S) was the original one and how one chromosome has been derived from the other.

If X_L was the original one, X_S might have been arisen by:

1. An intercalary deletion of X_L.
2. A crossing-over between X_L and Y_1 (normally no crossing-over in males).
3. A translocation between Y_1 and X_L.

If X_S was the original, X_L might have arisen by repeated duplication of the chromosome segment (or part of it) between centromere and SC of X_S (repetitive DNA).

For Dipteran species such polymorphisms have been recorded for instance in *Musca domestica* (Boyes, 1967; Milani et al., 1967) and in three different species of Tipulidae (Ullerich et al., 1964).

Chromosome Y_2 and Evolutionary Aspects

In various respects the small metacentric sex chromosome is different from the other chromosomes. On account of the absence of somatic and meiotic pairing with the other sex chromosomes and the fact that this chromosome is only present in some material, van Heemert (1974a) considered this chromosome to be a B-chromosome. From the cytological results of different hybridization experiments it can be concluded, however, that the small metacentric is a male determining chromosome. As mentioned before, initially only stocks with X_S chromosomes were available. Because chromosome Y_1 cannot be distinguished morphologically from X_S, the holandric inheritance of the metacentric (Y_2) could be explained in two ways: 1) the metacentric is a Y chromosome and both acrocentrics of the male parent are X chromosomes 2) one of the acrocentrics of the males is Y_1 and the holandric inheritance is caused by preferential segregation, i.e. Y_1 and the metacentric always go to the same pole.

However, using the long acrocentric X_L, it was possible to discriminate between the two alternatives by choosing as male parent an individual with a heteromorphic acrocentric pair. As shown in Table 3 there is no preferential disjunction of the metacentric with regard to one of the acrocentrics. Both acrocentric sex chromosomes of the male parent show a Mendelian inheritance, while the metacentric is again only found in the male offspring; there were no males without it. This can only be explained by assuming that both acrocentrics are X chromosomes (X_L and X_S) and the metacentric (Y_2) has a male determining function.

The frequently observed somatic numerical variation of chromosome Y_2 can bring about sex ratios significantly different from 1:1 (Table 1). Progenies

with an excess of males (57d, 69d, 75d) come from males with testes with a relatively high number of cells with $2Y_2$s and progenies with an excess of females (59d, 62d, 73d) come from males with testes mainly consisting of cells with 0 and 1 Y_2. More details will be presented elsewhere (Vosselman, in prep.)

Because chromosome Y_2 does not pair (meiotically and somatically) with chromosome X_S, while X_S pairs for the whole length with Y_1, it seems reasonable to assume that Y_2 has at most a very small segment homologous with Y_1. The absence of any pairing between a likely mutated, nonfunctional Y_2 chromosome and Y_1 (Vosselman, in prep.) leads to the same conclusion. However, judging from the sexual phenotypes the genetic information seems to be the same, in any case for the primary male characteristics. The most logical hypothesis to explain this, is that the male determining genes (or gene) are restricted to a very small chromosome segment and that this segment has been involved in a translocation (see below). In *Chironomus tentans* Beermann, 1955) and *Aphiochaeta xanthina* (Tokunaga, 1958; Mainx, 1959) two non-homologous sex determining chromosomes have been found. In both cases it is supposed that the two types have arisen by a translocation of the male determining gene(s). In polytene chromosomes of *Calliphora erythrocephala* a structural difference between the X and Y consists of a single additional intercalary heterochromatic band (Ribbert, 1967). It has not been proven, however, that the male determining gene(s) are indeed restricted to this additional chromosome segment. That sex determination can be restricted to a small chromosome segment or even to one locus has been shown e.g. for *Aedes aegypti* (McClelland, 1967) and for *Culex pipiens* (Gilchrist and Haldane, 1947).

It is still an open question which chromosome has been the original male determining chromosome. We will discuss two alternative hypotheses: 1) Y_2 is the original Y chromosome and Y_1 has arisen by a translocation between Y_2 and one of the X chromosomes. 2) Y_1 is the original one and Y_2 has arisen by a translocation between Y_1 and a supernumerary. There are some arguments which favour the hypothesis that Y_2 is the original Y chromosome. First, the observed low transmission by females of a nonfunctional form of chromosome Y_2 (Vosselman, in prep.) is an indication that this chromosome may not be able to function as a supernumerary, owing to an elimination from the population in a number of generations. This nonfunctional form of Y_2 has been observed in only one cross and is studied in crosses derived from this material. Secondly, from an evolutionary point of view it is more likely that a chromosome mutation $Y_2 \rightarrow Y_1$ has taken place, because Y_1 has a stable and Y_2 has an unstable somatic behavior. Except for gynandromorphs, it is not clear if a somatic numerical variation of Y_2 has indeed implications for fertility/fitness of the XXY_2 males. An argument in favour of the hypothesis that Y_2 originally has been a supernumerary may be, that a frequent occurrence of somatic nondisjunction is often reported for supernumeraries, especially in plants but also in some insect species (for references see Hewitt, 1973; White, 1973; Jones, 1975), and hardly for other chromosomes.

Boyes (1954) reported the possible presence of three morphologically different supernumeraries in *Hylemya cilicrura*. The inheritance of these chromosomes

is not known. The good agreement in respect of morphology and length of one of the supernumeraries with Y_2 of *H. antiqua* is striking. Therefore it seems worthwhile to investigate if this chromosome of *H. cilicrura* is indeed a supernumerary and not a Y chromosome, the more so as in this species also gynandromorphs and/or intersexes have been found (Sick, 1967). In *Hylemya fugax* (Boyes, 1952, 1954) and $X_1X_1X_2X_2/X_1X_2Y$ sex determination is present. Maybe a long supernumerary of *H. cilicrura* is involved in the derivation of the *fugax* complement (Boyes, 1952) or the *fugax* complement is derived from a 14 chromosome complement (Boyes, 1954).

The association of Y_2 with a particular segment of chromosome 4 in larval ganglion cells may indicate some kind of homology. However, the fact that no male determining genes have been detected in chromosome 4, the association has not been observed in meiotic cell stages and the derivation must have been rather complicated, it is more likely that there is some kind of nonhomologous association. Likewise it is questionable if the sometimes observed somatic association of the proximal part of X_L with chromosome 4 and with Y_2, is caused by homology. The disjunction of Y_2 in respect of X_L and X_S is random (Table 3).

About the relative frequencies of XY_1 and XXY_2 males and the distribution of these karyotypes in natural populations of the onion fly, only limited information is available. The material studied here originates from 24 males, randomly taken from a population, which had been collected in the Netherlands and reared during five generations in high numbers under laboratory conditions. From the karyotypes observed in progenies of these males, it could be concluded that 20 were XXY_2 and 4 were XY_1. Possibly XY_1 and XXY_2 also coexist in Canada. Van Heemert (1974a) has observed onion flies of a Canadian population with a small metacentric chromosome, morphologically identical with Y_2. Boyes (1954), however, has only described males with acrocentric sex chromosomes.

Gonadal Influence on Phenotype of Germ Cells and Gynandromorphism

In mammals much work has been done to establish if XX or XY cells can develop as germ cells in a phenotypically opposite gonadal environment. All results with tetraparental chimaeric mice (Mintz, 1968; Mystkowska and Tarkowski, 1968, 1970; Milet et al., 1972; McClaren, 1972, 1975; Ford et al., 1975) indicate that only XY cells in XX/XY male chimaeras can develop into functional sperm. The research done in cattle and marmoset monkeys has given conflicting results and this is discussed extensively by Ford and Evans (1977). In mouse (Evans, in Ford and Evans, 1977) and marmoset (Hampton, cited by Ford and Evans, 1977) XY oocytes have been observed and may even be functional. In *Drosophila* van Deusen (1976) has obtained evidence that donor cells in a host gonad of the opposite phenotypic sex cannot differentiate into functional gametes. In *H. antiqua* XX germ cells in an XX/XXY_2 (XXY_2Y_2)

mosaic testis can contribute to the functional spermatozoa of the males, as can be concluded from the following observations:

1. In several progenies from the cross between XX (♀) and XX/XXY_2 (♂), Table 1, and see Vosselman (in prep.), a significant excess of females has been observed.

2. XX cells from XX/XXY_2 chimaeric testis show male diplotene/diakinese/MI stages (Fig. 2g).

To what extent XXY_2 cells in ovarian tissue can develop into germ cells is not fully clear. The presence of some cysts with spermatozoa in ovaria of two gynanders may suggest that the "phenotypic sex" of an XXY_2 cell is determined by its own chromosome constitution. The lack of cytological information of oogonial divisions and the limited information about the distribution (patch size) of genotypically different cells in the somatic tissue of the ovaria of these gynanders, makes it impossible to draw more definite conclusions.

The occurrence of gynanders in the onionfly supports the generally accepted idea that in insects no circulating sex hormones are present (White, 1973). Hitherto only in larvae of the beetle *Lampyris noctiluca* strong indications have been found, that neurosecretory brain cells can play an endocrine role in the formation of apical tissue in the testis (Naisse, 1969). It is possible that in the onionfly within the testis Y-chromosome gene product(s) can diffuse, because XX cells can differentiate into male germ cells in a testicular environment. The presence of spermatozoa in the female gonad of some gynanders might be interpreted to support the hypothesis that within a gonad concentration differences for the Y-chromosome gene product(s) exist and that after exceeding a threshold for this product a cell can differentiate into a male germ cell.

One male without external genitalia has been found. Although this individual was the only one with an X_SY_2 constitution (at least in the testis) it is not likely that there is a causal relation between these two. Van Heemert (1974a) has observed phenotypically normal males without an X chromosome and with one Y_1 chromosome.

X-Polysomy

The results obtained with the X-polysomics show that Y_2 contains (an) epistatic male determining gene(s), which corresponds with the conclusion of van Heemert (1974b) for Y_1. Even $XXXXY_2$ individuals show no sign of intersexuality and have a normal spermatogenesis; no records about mating ability of this type are available. Males with an $XXXY_2$ constitution can mate and are apparently not strongly reduced in their fertility. The same remarks can be made for XXX females, and XXXX females are in any case viable. Rubini and Palenzona (1967) have also clearly demonstrated that additional X chromosomes do not affect sexuality of the housefly, even males with five X and one Y are fertile. However in the housefly also autosomal genes can play a role (Milani et al., 1967; Wagoner, 1969).

In several mammalian species, e.g. mouse (Cattanach, 1961; Russel and Chu, 1961; Slizynski, 1964), pig (Breeuwsma, 1968), sheep (Bruere et al., 1969),

dog (Clough et al., 1970) and tamnar wallaby (Sharman et al., 1970), it has been observed that an XXY constitution causes sterility. Also the Klinefelter syndrome in man is attended with complete azoospermia at the time of puberty (Ferguson-Smith, 1959; Mittwoch, 1967).

Normal spermatogenesis in $XXXXY_2$ males and the production of XX sperm in XX/XXY_2 chimeras are indications that spermatogenesis in the onionfly is regulated by another system. As outlined before, it is possible that exceeding a threshold for (a) Y-chromosome gene product(s) is enough for "masculinization" of a future male germ cell and that this process is not influenced, or cannot be reversed by "any" number of X chromosomes.

The disjunction of the X-quadrivalent (in $X_LX_LX_LX_SY_2$ and $X_LX_LX_SX_SY_2$ males) seems to be very regular, all 62 scored MII cells contained 2 Xs.

This agrees with the observed regular orientation in diakinesis/MI, where the centromeres are directed two by two to either of the poles. The number of individuals scored in the progeny of two X-trisomic parents (Table 5) is not high enough to draw definite conclusions about the coorientation between X_S and X_L. It is only clear that there is no exclusive coorientation between fully homologous X-chromosomes.

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Sex Determination of the Onion Fly, *Hylemya antiqua* (Meigen)

II. Sex Ratio Distortion by Unstable Somatic Behaviour of Chromosome Y_2, and Inheritance of a Nonfunctional $Y_2(Y_m)$

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Abstract. In testes of many XXY_2 and XXY_2Y_2 males of *Hylemya antiqua* a numerical variation for Y_2, a consequence of mitotic nondisjunction, has been established. In 22 of 79 progenies of XXY_2 (including some unidentified XXY_2Y_2) males the ratio of females to males was significantly ($p < 0.05$) different from 1:1, 9 with excess females and 13 with excess males. It could be concluded, firstly, that in progenies of XXY_2 males sex ratio distortion results from a numerical variation of Y_2 between the primordial germ cells of the male parent and secondly, that probably at least three primordial germ cells are involved in the formation of the male germ cells of the onion fly. Besides, the occurrence of some highly distorted sex ratios in male direction could be attributed to the presence of XXY_2Y_2 males. The frequency of M II cells with 2 Y_2 chromosomes established in XXY_2 males amounted to 2.9%. In XXY_2Y_2 males this percentage was higher and occasionally M IIs with 3 Y_2 were found. In the progeny of one cross a chromosome morphologically identical with Y_2 but lacking its holandric inheritance was found. From its inheritance, it could be concluded that it was a nonfunctional (mutated) Y_2 chromosome (Y_m). Interesting phenomena were the reduced transmission of Y_m through the female, and the observation of very high numbers of Y_m in some ovarian cells. A possible causal relationship between these two phenomena is discussed.

Introduction

Distortion of the sex ratio in favour of males, as a result of meiotic drive or by combination of certain sex determining factors, can be useful for genetic control of insects.

A well-known example of meiotic drive resulting in sex ratio distortion is the distorter gene in *Aedes aegypti* (for references, Newton et al., 1976). Results of field cage experiments with sex ratio distorter-translocation heterozygotes (Suguna et al., 1977a) have shown that a practical application might

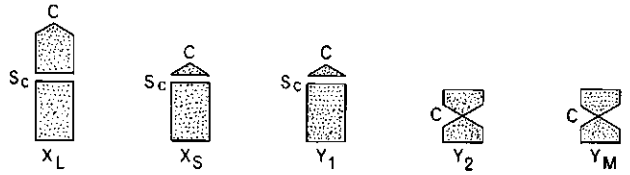

Fig. 1. Sex chromosomes of *H. antiqua*, diagrammatically. Y_m is probably a nonfunctional Y_2. *C* centromere, *Sc* secondary constriction

be possible, although this can be hampered by the occurrence in certain strains of "resistance" to the expression of sex ratio distortion (Wood, 1976; Suguna et al., 1977b).

The observation of sex ratios significantly different from 1:1 in certain crosses of the onion fly, *Hylemya antiqua*, initiated a project to determine the genetics of this sex ratio distortion and its possibilities for an eventual practical application in a genetic control program. Some of these results have been presented earlier (Vosselman, 1978). The most important observations were:

1. The existence of a polymorphism for the acrocentric X-chromosome in a native Dutch population of the onion fly. It concerns a difference in length of the chromosomes, the short one being called X_S and the long one X_L (Fig. 1).

2. In the same Dutch population XY_1 and XXY_2 males coexist. The acrocentric Y_1 is homologous and morphologically identical with X_S. On the contrary, Y_2 is metacentric and does not pair with the X-chromosomes (Fig. 1).

3. The occurrence of sex ratio distortion in progenies of $XXY_2(Y_2)$ males. This was attributed to a numerical variation for Y_2.

The present paper deals with this numerical variation of Y_2 and the sex ratios in progenies of $XXY_2(Y_2)$ males. Further attention will be paid to the inheritance of a chromosome (Y_m), morphologically indistinguishable from Y_2 but without the holandric inheritance of Y_2. The inheritance of this chromosome has been studied primarily to check the possibility that it is a true Y_2, but that its male determining strength is overruled by an epistatic female determining gene. Such a case has been found in *Musca domestica* by Milani et al. (1967) and by crossing certain genotypes of *M. domestica* it was possible to produce all male progenies (Milani, 1971; Wagoner et al., 1974).

Material and Methods

Chromosome Y_2. For rearing conditions and cytological techniques see Vosselman (1978).

The flies used for determining the sex ratios and somatic numerical variation of Y_2, came all from $X_SX_S(♀) \times X_SX_SY_2(Y_2)$ (♂) crosses. Chromosome X_S is further indicated as X. To save rearing work, these experiments were carried out partly in combination with a program for the isolation of translocation homozygotes for T14 (subject for other papers). Therefore in some crosses T14 was present, a reciprocal translocation between the autosomes 2 and 6. It has no impact on the sex ratios. For determining the sex ratios the following crosses were excluded. Those with: (1) a population percentage (number of pupae divided by the number of hatched eggs × 100) lower than 80; (2) an egg mortality higher than 5% (except in crosses with translocation heterozygotes) and (3) a pupal mortality higher than 10%.

For about half of the crosses (Table 1a and b) single pairs were used. In six cases one male had mated with two females. Because no significant differences in sex ratios were observed among the progeny, in all six cases the results of both females have been combined. The remaining results were obtained from females from mass cages containing on average 10 males and 10 females, which had been together for 1–3 days at an age of 10–14 days. From unpublished data it was concluded to be unlikely that the females of such mass cages have mated more than once, but multiple matings of males could not be excluded.

Chromosome Y_m. For studying the inheritance of chromosome Y_m the flies were crossed according to the scheme of Figure 3. In progeny D3-1 some females with a small metacentric chromosome (Y_m) morphologically identical to Y_2 were observed for the first time. The parental karyotypes of D3-1 were not known. The mother came from a backcross-program for T14 and the male parent from a native Dutch population (this population was maintained during 5 generations under laboratory conditions).

The XXY_m females of D3-1 were crossed with XY_1 males (i.e., without the metacentric) and the XXY_2 males with XX females (Fig. 3). In the following generation (G2) the males of progeny Id-6 (XY_1 or XY_1Y_m) were crossed with XX females. These females came from two $XX(♀) \times XXY_2(♂)$ crosses, from which it was known that Y_2 was inherited in a strictly holandric way.

In the first generation G_1 (Table 3) no specific attention was paid to the numerical variation of the small metacentric (Y_m and/or Y_2). When the majority of cells of an individual contained more than 1 Y_m/Y_2 it was classified as 2 Y_m/Y_2. For the classification of the individuals in the next generation G_2 (Table 4) the following procedure was used:

1. Individuals with a mean number of Y_m chromosomes per cell falling in the range 0.01–1.50 and with as highest relative frequency either the class 0 Y_m or 1 Y_m, were classified as 1 Y_m.
2. Individuals with a mean number of Y_m falling in the range 1.51–2.50 and with as most frequently occurring cell class 2 Y_m, were classified as 2 Y_m.
3. All other individuals with chromosome Y_m which did not meet the criteria mentioned above, have been called var. Y_m. It concerns mainly individuals with very divergent numbers of Y_m per cell.

The number of scorable cells in females varied from 5–15, in larvae from 10–25 and in males the average number was higher than 20.

The transmission rate of Y_m is defined as the number of individuals in the offspring multiplied by the respective numbers of Y_m, divided by the total number of individuals.

Results

Chromosome Y_2

In Table 1a is given the frequency distribution of the sex ratios of 79 progenies of XXY_2 (and XXY_2Y_2) males, which were crossed with XX females. The average number of flies per progeny was 82.5 and the minimum number 41. In total 3078 females and 3436 males were observed. For the crosses with ratios of females to males not significantly different from 1:1 these numbers were 2275 and 2235 respectively. Gynandromorphs, at least the recognizable ones, have been left out of consideration.

Of the 79 progenies given in Table 1a, 22 had a ratio of females to males significantly different from 1:1 ($p < 0.05$), 9 with excess females and 13 with excess males (Table 1b). For the progenies with excess females in all cases a ratio had been observed agreeing well with ♀:♂ = 2:1 (Table 1b). Even more distorted ratios in favour of females were found in another experiment. In the progeny of two females (which had mated very likely with the same male) 62 females and 4 males, and 42 females and 6 males respectively were present.

Table 1.

a Frequency distribution of sex ratios in 79 progenies of $XXY_2(Y_2)$ males of the onion fly. The number of individuals per progeny was variable, but always more than 40. See also **b**

Sex ratios (no. ♀♀/ total no. of adults)								
0–0.10	0.11–0.20	0.21–0.30	0.31–0.40	0.41–0.50	0.51–0.60	0.61–0.70	0.71–0.80	0.81–1.0
1	5	1	5	28	27	11	1	0

b Numbers of females and males of progenies of $XXY_2(Y_2)$ males with a sex ratio significantly different from 1:1 (χ^2's; $p<0.05$), c.f. **a**. Only for progenies 1–9 and 12–16 expected numbers are given (see text)

Excess of females			Excess of males					
Progeny code	No. of		Progeny code	No. of		Progeny code	No. of	
	♀♀	♂♂		♀♀	♂♂		♀♀	♂♂
1	54	27	10	46	67	17	22	128
2	30	13	11	67	95	18	22	121
3	71	31				19	15	75
4	55	31				20	10	52
5	53	30	12	52	103	21	5	41
6	49	18	13	26	60	22	7	86
7	53	32	14	33	65			
8	32	18	15	17	36			
9	68	37	16	16	35			
Total (1–9)	465	237	Total (12–16)	144	299			
Exp. (2:1)	468.0	234.0	Exp. (1:2)	147.7	295.2			

For the progenies containing excess males, different ratios were observed (Table 1b). Progenies 12 to 16 fit a ratio of ♀:♂ = 1:2, but the remaining ones (except 10) are significantly different from 1:2. Probably the male parents of progenies 17 to 22 were 2 Y_2 (see discussion) and for these no expected numbers are given.

The cytological results of 118 randomly chosen $XXY_2(Y_2)$ males in respect of the numerical variation for Y_2 in gonadal cells are given in Table 2. Only males with at least 10 scorable cells, spermatogonial metaphases and cells in diakinesis-metaphase I, have been used. The number of cells scored per male was variable. In general spermatogonial metaphases are better suited for determining the presence and number of Y_2 chromosomes, because somatic pairing is not as strong as meiotic pairing. Consequently, in spermatogonial metaphases there is always some distance between the (autosomal) homologues, so the chance that a Y_2 chromosome was covered by another chromosome and hence not detected is not as high as for diakinesis/MI. Further, the percentage of broken cells seems to be somewhat higher for diakinesis/MI and possibly the Y_2 chromosome, because of its small size, is lost more easily than the other chromosomes. This might explain the difference between these cell types for the frequency distribution of the Y_2 chromosome (Table 2).

Table 2. Numerical variation of Y_2 in testes of $XXY_2(Y_2)$ males of *H. antiqua*. The males have been grouped according to the mean number of Y_2s. The percentages of cells with a particular number of Y_2s are averages of all males in the corresponding range

Range of mean no. of Y_2/δ	No. of males cells	Mean no. cells scored/δ	Percent cells with the no. of Y_2 chromosomes			
			0 Y_2	1 Y_2	2 Y_2	3 Y_2
0.20–0.39	3	20.0	71.7	26.7	1.7	0.0
0.40–0.59	6	23.8	60.1	30.8	6.3	2.8
0.60–0.79	14	22.3	50.3	39.1	8.7	1.9
0.80–0.99	28	27.2	22.6	66.5	9.7	1.2
1.00–1.19	40	22.9	9.2	78.8	10.8	1.2
1.20–1.39	7	22.6	11.4	50.6	33.5	4.4
1.40–1.59	10	26.6	9.8	40.2	38.7	11.3
1.60–1.79	3	26.0	19.2	10.3	51.3	19.2
1.80–1.99	5	16.6	2.4	20.5	65.1	12.0
2.00	2	10.0	5.0	10.0	40.0	45.0
sum and means	118	23.7	21.6	58.1	16.7	3.6
Spermatogonial metaphases (n = 1630)			19.9	57.8	18.2	4.1
Diakinesis/MI's (n = 1168)			24.0	58.5	14.6	2.9

A spermatogonial metaphase with 2 Y_2 and one with 3 Y_2 are shown in Figure 2a and b respectively. When higher numbers of Y_2 were present it was not always possible to determine the exact number, particularly in diakinesis/MI stages but also in spermatogonial metaphases (Fig. 2c). Therefore cells with 3 and more Y_2s have been treated as one group (Table 2).

The males in Table 2 have been grouped according to the ranges into which their mean number of Y_2s fell. Within a range the frequency distribution (in%) has been calculated on the basis of all cells of all males belonging to this range. It was observed that the frequency distribution of the males in the range 1.0–2.0 Y_2 was not bimodal or discontinuous (Table 2). Therefore, not all males could be classified as either a 1 Y_2 or a 2 Y_2 individual.

To obtain an estimate for the frequency of 2 Y_2 gametes, 715 metaphase II cells from 38 XXY_2 males were scored: 336 had 0 Y_2, 358 had 1 Y_2 and 21 had 2 Y_2. All these males had an average number of Y_2 in spermatogonia and spermatocytes lower than 1.3.

In five XXY_2Y_2 males (average number of Y_2 in diploid cells more than 1.5 and in MIIs about equal to 1.0) the following MII scores were obtained: 19 with 0 Y_2, 58 with 1 Y_2, 10 with 2 Y_2 and 3 with 3 Y_2. Further an offspring of a likely XXY_2Y_2 male has been analysed: 10 embryos had only cells with 0 Y_2, 41 had only or predominately cells with 1 Y_2 and in 9 embryos only or predominately cells with 2 Y_2 were found. In one embryo 8 cells with 4 Y_2 and 3 cells with 3 Y_2 were observed. A case of nondisjunction of Y_2 in an embryo is shown in Figure 2d, 3 Y_2 chromosomes go to one pole and 1 Y_2 to the other.

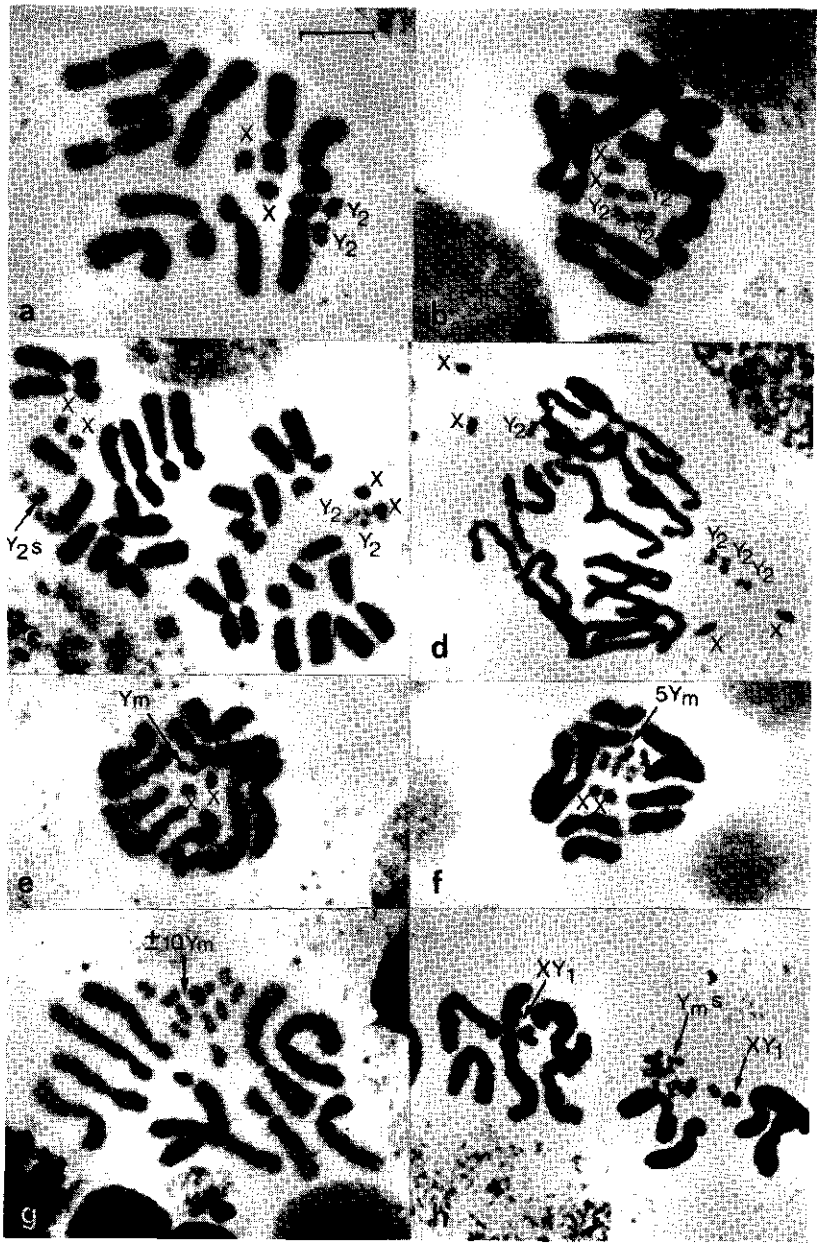

Fig. 2a–h. Different numbers of Y_2 and Y_m chromosomes in *H. antiqua*. **a** Spermatogonial metaphase with 2 Y_2. **b** Spermatogonial metaphase with 3 Y_2. **c** Spermatogonial metaphase with 2 Y_2 next to one with 4 or 5 Y_2. **d** Somatic anaphase in young embryo with nondisjunction of Y_2 (3 $Y_2 \leftrightarrow 1$ Y_2). **e–g** Somatic metaphases in ovaria with 1 Y_m, 5 Y_m and ± 10 Y_m respectively. **h** Diakinesis/prometaphase. One cell with cluster(s) of Y_m chromosomes and one with 0 Y_m. Bar represents 10 μm

Generation	Progeny code	Karyotypes		Remarks
		Females	Males	
G_0		XX ——— × ———	XXY_2Y_m	Hypothetical, on basis of progeny
G_1	D3-1	XXY_m	XXY_2	See Table 3. Results of testcrosses of males, see text
			XY_1	
G_2	Ia-4 Ia-5 Id-6	XX XXY_m	XY_1 XY_1Y_m	See Table 4
		XX ——— × ———		
G_3	Ibd/2 Ibd/6	XX XXY_m	XY_1 XY_1Y_m	See Table 5. Progenies of XY_1 males are not given here

Fig. 3. Crossing scheme for chromosome Y_m of *H. antiqua*. Karyotypes present in low frequencies have not been presented here

Table 3. Frequencies of karyotypes observed in progeny D3 1 (see Fig. 3). Because Y_2 and Y_m cannot be distinguished morphologically, a separate classification for these chromosomes was not possible ($1Y_2/Y_m$ means either Y_2 of Y_m, $2\ Y_2/Y_m$ means either Y_2Y_m or Y_2Y_2 or Y_mY_m)

Stage of classification	No. of individuals with		
	$0\ Y_2/Y_m$	$1\ Y_2/Y_m$	$2\ Y_2/Y_m$
eggs	1	12	2
larvae	1	19	4
females	1	4	0
males	0	4	1
total	3	39	7
Exp.[a]	12.3	24.6	12.3

[a] Expected numbers if the parents were XXY_m (♀) and XXY_2 (♂) in absence of a numerical variation for Y_m and Y_2

Chromosome Y_m

In one progeny D3-1 (Fig. 3) of 39 individuals (eggs and young larvae) 31 were found to be mono-, 6 di- and 2 nullisomic for a small metacentric chromosome (Table 3), initially thought to be the strictly male determining chromosome (Y_2). Therefore a highly distorted sex ratio was expected. But surprisingly the ratio of females to males in the remaining individuals of this cross was not

Table 4. Karyotypes and numbers of females and males in progenies of XXY_m (♀) × XY_1 (♂), testcrosses of females of D3-1 (see Fig. 3). Only the results for Y_m have been mentioned, because X and Y_1 cannot be distinguished morphologically. Larvae cannot be sexed. Some individuals with a numerical variation for Y_m were classified as var. Y_m, for explanation and criteria see text

Progeny number	Date egg rafts (1976)	Stage/ sex	No. of individuals with				No. of	
			0 Y_m	1 Y_m	2 Y_m	var. Y_m	♀♀	♂♂
Ia-4	2/12	larvae	22	6	1	0		
Ia-4	10/12	females	25	5	1	1		
Ia-4	10/12	males	11	7	2	1		
Ia-4		total	58	18	4	2	38	21
Ia-5	2/12	larvae	22	8	2	3		
Ia-5	10/12	larvae	40	4	0	2		
Ia-5		total	62	12	2	5	–	–
Id-6	2/12	larvae	15	8	0	0		
Id-6	6/12	females	12	6	0	3		
Id-6	6/12	males	14	7	2	0		
Id-6	16/12	larvae	44	0	0	3		
Id-6		total	85	21	2	6	46	58
total (all crosses)			205	51	8	13		

significantly different from 1:1 and in four of five females scored, a small metacentric was present (Table 3). This metacentric chromosome present in females also showed an unstable mitotic behaviour and was morphologically identical with Y_2 (compare Fig. 2, a–d with Fig. 2, e–h).

The unexpected presence of this chromosome in females might be interpreted as follows:

1. It is a mutated, non-functional Y_2 chromosome, i.e. it has lost its male determining function and is called Y_m.

2. It is a Y_2 chromosome, but an epistatic female determining factor, prevents male expression.

3. It is a B-chromosome. Y_2 has been derived from it by translocation of the small male determining segment of Y_1.

To exclude some of these hypotheses further crosses with this material were made.

Firstly the males from G_1 (Fig. 3) were testcrossed with XX females. Males and females in three progenies were karyotyped. In all 37 males scored the small metacentric was present, while it was absent in all 35 females scored. Apparently the three testcrossed males had a XXY_2 karyotype (and not XY_1Y_m), because the metacentric chromosome (Y_2) was inherited in a strictly holandric way.

Secondly, females from G_1 (Fig. 3) were testcrossed with XY_1 males, the

Table 5. Karyotypes and numbers of females and males in progenies of males from cross Id-6 (Fig. 3). These males, either XY_1 or XY_1Y_m, were crossed with XX females. The results of two progenies with Y_m karyotypes have been presented separately. Chromosomes X and Y_1 cannot be distinguished morphologically

Progeny code	Females		Males		No. of	
	0 Y_m	1 Y_m/2 Y_m	0 Y_m	1 Y_m/2 Y_m	♀♀	♂♂
I bd/2	3	2	7	3	101	90
I bd/6	3	12	4	6	88	66
Other crosses (8×)	5	0	55	0	248	258

results are given in Table 4. In two progenies (Ia–4 and Id-6) adults were karyotyped and in both progenies males and females with and without chromosome Y_m were found.

In progeny Id-6 there was no significant difference between the sexes in respect of the number of individuals with and without Y_m. On the contrary, in Ia-4 relatively more males than females with Y_m were found (Contingency $\chi_1^2 = 3.9$; $0.02 < p < 0.05$). Another unexpected observation was the occurrence of almost twice as many females as males in Ia-4 (χ_1^2 (1 : 1) = 4.9; $0.02 < p < 0.05$). No more adults were available to confirm these results.

Many individuals showed again a somatic numerical variation for Y_m. A remarkable observation was the rather frequent occurrence of mitotic dividing ovarian cells with very high numbers of Y_m (Fig. 2, f–g), while in testes such cells only very occasionally were observed. Sometimes it was even impossible to determine the exact number (Fig. 2h).

Assuming all three female parents of the progenies mentioned in Table 4 were monosomic for Y_m as zygotes, one would expect a transmission rate of Y_m equal to 0.5. However, the observed average transmission rate of all progenies amounted to only 0.31, with the assumption that half of the individuals classified as var. Y_m were monosomic and half disomic. Apparently there is a loss of Y_m chromosomes, when transmitted through the female. In progenies Ia-5 and Id-6, but not in Ia-4, there were significant differences in number of individuals with and without Y_m between egg batches. The contingency χ^2s were 6.4 (1 d.f.; $0.01 < p < 0.02$), 15.6 (2 d.f; $p < 0.001$) and 0.6 (1 d.f.; $0.30 < p < 0.50$) respectively. Both in Ia-5 and Id-6 in the last egg batch a very low number of individuals with Y_m were observed.

With the results obtained in generations G_1 and G_2 (Tables 3 and 4) it was still not possible to discriminate between the three hypotheses mentioned above. Chromosome Y_m might be a Y_2 chromosome, if a female determining gene epistatic to Y_2 and Y_1 was present. Therefore males in progeny Id-6 (G_2) either XY_1 or XY_1Y_m (Fig. 3) were testcrossed again with XX females. The results are given in Table 5. In only two progenies was chromosome Y_m present. In neither cross was there a holandric inheritance of Y_m. In the eight remaining progenies no individual with Y_m was observed. The individual sex ratios of these progenies were not significantly different from 1 : 1.

Discussion

Chromosome Y_2

Sex ratio distortion in the onion fly is bidirectional, e.g., crosses with excess females and excess males occur (Tables 1a and b). The total number of males (3436) from all crosses was significantly ($p < 0.001$) higher than the number of females (3078). This excess of males is caused by a higher number of crosses with an excess of males together with a larger average number of individuals from such crosses. It is not likely that the numerical variation for Y_2 is linked with some kind of accumulation mechanism, as has been observed often for B-chromosomes with an unstable behaviour (s.f. Müntzing, 1974; Jones, 1975). Further consideration of the sex ratios observed is given below.

The numerical variation for Y_2 in the testes (Table 2) was established, to determine if a correlation between the observed and "expected" sex ratios was there. The "expected" sex ratios might be calculated as follows: number of cells with 0 Y_2 plus half of the number of cells with 1 Y_2 divided by the total number of cells per male. There are, however, two important disturbing factors.

Firstly, the average number of scorable cells per male was low, consequently random fluctuations can strongly affect the calculated average number of Y_2 per male. Secondly, the cells scored in the testes do not supply a random sample from the whole germinal cell population of a male. As meiotic and also mitotic divisions take place synchromously per cyst (Theunissen, 1976), the cytological data of a male in general will represent only a restricted number of cysts. The real number is not known because separate cysts could not be recognized. With a numerical variation for Y_2 between cysts a bias is easily introduced.

Taking this into consideration, it is not surprising that the number of progenies with aberrant sex ratios expected on account of the cytological data in the testes (Table 2) is higher than the observed number (Table 1a).

In some grasshopper species with an intra-individual numerical variation for B-chromosomes, the numerical variation in the testes is usually restricted to differences between follicles. Within follicle or cyst, the number is usually constant (Nur, 1963, 1969).

Because often neighbouring cells with different numbers of Y_2 chromosomes were observed, it seems probable that for Y_2 an intra- as well as an inter-cyst variation can occur.

Another conclusion from the data of Table 2 is that the frequency distribution of the males in the range 1.0–2.0 Y_2 is not discontinuous or bimodal, as would be expected. Consequently not all males can be classified as either 1 Y_2 or 2 Y_2. It is obvious that this will be partly due to the low average number of cells scored per male and the "non-randomness" of these cells (see above).

The average percentage of spermatogonia and spermatocytes with 3 or more Y_2 chromosomes was 3.6 (Table 2). Consequently a frequency of 2 Y_2 gametes of at least 1.8% was expected. A comparable value (2.9%) was found in MII cells from XXY_2 males. As expected a higher percentage of MII cells with $2Y_2$ was observed in XXY_2Y_2 males. On the basis of these estimated percentages

of 2 Y_2 gametes one would expect that the fathers of approximately two from the 79 progenies given in Table 1a would have been XXY_2Y_2. In fact 6 progenies (17–22) give indications that they had such a father.

A male having exclusively primordial germ cells with 1 Y_2 will not give a highly distorted sex ratio when the numerical variation for Y_2 occurs only as a result of nondisjunction in spermatogonia. To obtain a ratio of ♀:♂ = 2:1, $^2/_3$ of the gametes should have been 0 Y_2, $^1/_6$ 1 Y_2 and $^1/_6$ 2 Y_2 (average number of Y_2/gamete is 0.5). Consequently half of the male progeny should be XXY_2Y_2, but this has never been observed. On the contrary in a progeny of a male with exclusively 2 Y_2 primordial germ cells one can expect a highly distorted sex ratio only as a consequence of nondisjunction of Y_2 in spermatogonial divisions. The results observed in one offspring with 10 nulli-, 41 mono-, 9 di- and 1 tetra- or trisomic(s) for Y_2, form a good illustration. Very likely the male parent was XXY_2Y_2 and the presence of nulli and disomic embryos is the result of nondisjunction of Y_2 in spermatogonial divisions. However, to explain a ratio of ♀:♂ = 1:2 (supposing the male parent had only 2 Y_2 primordial germ cells) half of the male offspring had to be disomic for Y_2. This has never been observed.

As it is not possible to explain certain ratios, assuming that all primordial germ cells have the same number of Y_2, it is necessary to look for an explanation in terms of random drift. Nondisjunction of Y_2 in a spermatogonial division and a contribution to the germ cell population of only one daughter cell, might result in such a drift. However, a clearly measurable effect on sex ratio will be only possible when this takes place very early in spermatogenesis, during a very restricted number of cell divisions. Therefore, it is much more likely that sex ratio distortion (by drift) will be caused by a numerical variation for Y_2 between primordial germ cells. From *Drosophila* it is known that usually three to seven cleavage nuclei penetrate the pole region to become potential primordial germ cells (Sonnenblick, 1950). However, the actual number of cleavage nuclei involved in the formation of the primordial germ line might be lower (Lee et al., 1967). Supposing a germ cell primordium of the onionfly arises in a comparable way, and in every cell division there is a certain probability for nondisjunction of Y_2, the chance for a numerical variation for Y_2 between primordial germ cells will depend on the number of divisions between the "stem cell" and the primordial germ cells. "Stem cell" meaning here the cell from which all primordial germ cells have descended (not necessarily the zygote, unless this is the only common diploid cell). When nondisjunction of Y_2 occurs in one of the cell divisions between the "stem cell" and the primordial germ cells this will always result in a numerical variation for Y_2 between the primordial germ cells and in many cases it will also result in drift (if the average number of Y_2 is not equal to 1).

In Table 6 expected ratios of females to males (only those with an excess of females) for different numbers of primordial germ cells and with different numbers of Y_2 chromosomes for each primordial germ cell, are given. For simplification it is assumed that no 2 Y_2 gametes are formed, i.e., no spermatocytes with more than 2 Y_2 and no nondisjunction in meiosis. Other assumptions are: (1) an equal contribution of each primordial germ cell to the germ cell population, (2) no selection between gametes and no selection in (post)-zygotic

Table 6. Expected ratios of females to males in progenies of XXY_2 males with a numerical variation for Y_2 between primordial germ cells. Only the ratios with an excess of females, for different numbers of primordial germ cells, are given. For assumptions made, see text. 001 means two primordial germ cells with 0 Y_2 and one with 1 Y_2; 002 means two primordial germ cells with 0 Y_2, and one with 2 Y_2, etc.

Number of primordial germ cells	Expected ratios of females to males								
	9:1	7:1	5:1	4:1	3:1	7:3	2:1	5:3	3:2
2	–	–	–	–	01	–	–	–	–
3	–	–	001	–	–	–	011 002	–	–
4	–	0001	–	–	0011	–	–	0111 0012	–
5	00001	–	–	00011 00002	–	00111 00012	–	–	01111 00022

stages. With these assumptions for instance in progenies of 011 males (three primordial cells, one with 0 Y_2 and two with 1 Y_2) a ratio of ♀:♂=2:1 is expected. The same ratio is expected for a 002 male, because one third of the spermatocytes will be 2 Y_2 and these will produce only 1 Y_2 gametes.

All ratios (and the average one) with an excess of females (Table 1 b) agree well with a ratio of ♀:♂=2:1 and this would correspond with 3 primordial germ cell, but a higher number of primordial germ cells can certainly not be excluded (Table 6). For two progenies, originating very likely from the same male parent, ratios significantly different from 2:1 were observed. The total number of females and males of both progenies were 104 and 10 respectively. This ratio agrees well with 9:1 or 7:1, which corresponds to 5 or 4 primordial cells, but is significantly different from 5:1 ($\chi_1^2=5.0$; $0.02<p<0.05$).

Progenies of XXY_2Y_2 males cannot be used for estimating the number of primordial germ cells. For example a ratio of ♀:♂=1:5 is possible when the male parent had (1) two primordial germ cells with 2 Y_2 and one with 1 Y_2 but also when (2) all primordial germ cells had 2 Y_2 but as a consequence of nondisjunction in spermatogonial divisions in 17.7% of the gametes 0 Y_2 were present. Only for progenies 10 to 16 (Table 1 b) is it practically certain that the male parent was an XXY_2 individual (as a zygote). Progenies 12 to 16 fit well to a ratio of ♀:♂=1:2, although some other ratios cannot be excluded, but progeny 11 is significantly different from 1:2 ($\chi_1^2=4.7$; and fits better to a 3:5 or 2:3 (4 or 5 primordial cells). Progeny 10 fits better to a 3:5 or 2:3 but is not significantly different from 1:2.

The male parents of progenies 17 to 22 were probably all XXY_2Y_2 but it cannot be excluded that some originate from a XXY_2 zygote.

Summarizing, it is likely that sex ratio distortion is caused by a numerical variation for Y_2 between primordial germ cells, and that at least three primordial cells are involved in the formation of the male germ cell population of the onion fly. A more practical conclusion is that the sex ratio distortion caused by the unstable somatic behaviour of Y_2 can not be used for a genetic control of the onion fly. There are no possibilities for an efficient selection of a population with an excess of males.

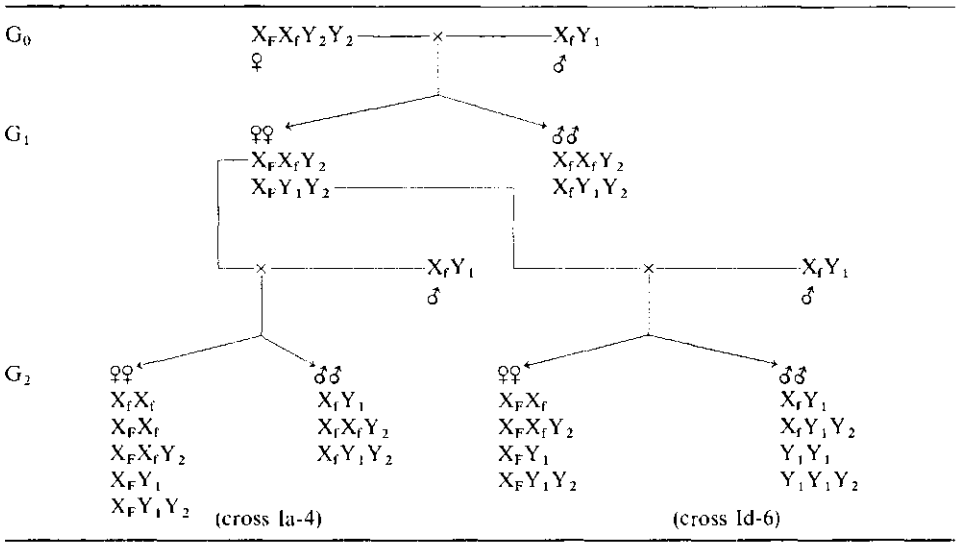

Fig. 4. Hypothesis concerning a female determining, X-linked gene (indicated as X_F, X_f is normal X), which is epistatic to Y_1 and Y_2. See Figure 3

Chromosome Y_m

The frequency distribution of individuals with 0, 1 and 2 small metacentrics (Y_2 and/or Y_m) in progeny *D*3-1 (Table 3) indicated that (1) the parental karyotypes were XX(♀) and XXY_2Y_m(♂) and (2) there was a coorientation between Y_2 and Y_m. The presence of some individuals with 0 and 2 small metacentrics will be the result of a numerical variation in the male parent. If the parents were either XXY_m(♀) and XXY_2(♂) or XX(♀) and XXY_2Y_m(♂) but without a coorientation between Y_2 and Y_m then, in absence of numerical variation, the expected frequencies with 0, 1 and 2 small metacentrics among 49 flies would be 12.3, 24.6 and 12.3 respectively. The observed numbers, however, are significantly different ($\chi_1^2 = 17.8$; $p < 0.001$). With a numerical variation for Y_2 and Y_m the expected numbers can not exactly be calculated, but also then significant differences are expected. A coorientation between Y_2 and Y_m indicates that these chromosomes are homologous and not just morphologically identical.

With the data obtained in G_1 and G_2 (Tables 3 and 4) it could not be excluded that the small metacentric present in females was a Y_2 chromosome, if an X-linked female determining factor (X_F, with X_f as normal X chromosome) epistatic to both Y_2 and Y_1 was present (Fig. 4). In that case the parents of progeny Ia-4 would have to be $X_FX_fY_2$(♀) and X_fY_1(♂) to explain the excess of females and the difference between the sexes in number of individuals with and without Y_2 in this progeny (Fig. 4). The 1:1 ratio of females to males in progeny Id-6 could be explained if its parents were $X_FY_1Y_2$(♀) and X_fY_1(♂). It was not possible to establish cytologically if Y_1 was present in the females, as Y_1 is morphologically identical to the X chromosome. To verify this hypothesis, males of progeny Id-6 (for different genotypes see Fig. 4) were crossed with XX females from which was known on the basis of other crosses that they did not have an epistatic female determining gene.

The results (Table 5) falsify this hypothesis. For example in an $X_fX_f(♀) \times X_fY_1Y_2(♂)$ cross one would expect a holandric inheritance of Y_2 with an excess of males, and in an $X_fX_f(♀) \times Y_1Y_1Y_2(♂)$ cross one would expect, only males: neither of these was observed. Likewise, these observations do not fit other hypotheses concerning female determining gene(s), epistatic to Y_2 (and Y_1). So probably this metacentric chromosome is a nonfunctional Y_2 chromosome. Another possibility might be that Y_m is a B-chromosome from which Y_2 has been derived by means of a translocation of the male determining segment of Y_1. But from an evolutionary point of view this is not very likely, because of the low transmission of Y_m through the female (see below) it is not expected that Y_m can maintain itself in a population during a period long enough to permit this development.

The difference (contingency $\chi_1^2 = 3.9$; $0.02 < p < 0.05$) between the sexes in respect of presence and absence of chromosome Y_m in progeny Ia-4 is difficult to explain. It is possible that it is partly due to the generally lower number of scorable cells and a higher numerical variation of Y_m in females. Likewise it is not clear why the sex ratio of cross Ia-4 is significantly different from 1:1 ($\chi_1^2 = 4.9$; $0.02 < 0.05$). In many other crosses with chromosome Y_1 as the male determining chromosome no aberrant sex ratios were observed.

A remarkable observation was the rather frequent occurrence of mitotically dividing ovarian cells with more than 4 Y_m chromosomes. Since these cells were mostly found together with cells with 0 and 1 Y_m but without intermediate numbers it is suggested that once nondisjunction starts, it is continued in several cell generations, perhaps as a result of stickiness. Further, as in males only rarely cells with more than four Y_m (or Y_2) chromosomes were found, there seems to be a specific interaction between the ovarian tissue and Y_m, resulting in such a recurrent nondisjunction.

When these cells with high numbers of Y_m do not contribute to the effective female gamete population, which is not unlikely as never individuals with on average more than 2 Y_m were observed, a loss of Y_m chromosomes is expected. This would correspond with the low observed transmission of Y_m through females, but the variation between egg batches in progenies Ia-4 and Id-6 is difficult to explain in this way. Another possibility might be some kind of meiotic drive. In insects several meiotic drive and also pre- and postmeiotic mechanisms have been suggested and could be demonstrated to be the reason of a non-mendelian inheritance of a chromosome (reviews by Zimmering et al., 1970; Müntzing, 1974; Jones, 1975).

From the inheritance of Y_m and the similarities in morphology and unstable somatic behaviour between Y_m and Y_2, it was concluded that Y_m is a nonfunctional Y_2 chromosome. Therefore, to produce all male progenies by crossing certain genotypes as in *M. domestica* (Milani, 1971; Wagoner et al., 1974) does not seem to be possible with the sex determining system of *H. antiqua* studied here.

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A MALE-LINKED TRANSLOCATION WITH HIGH FERTILITY IN THE ONION FLY HYLEMYA ANTIQUA (MEIGEN)

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A male-linked translocation has been isolated following irradiation with fast neutrons. The translocation breakpoints in chromosome 2 and Y are in the vicinity of the centromeres. Predominantly alternate segregation occurred which explains the high fertility. The use of male-linked translocations for a genetic sexing method is discussed.

Introduction

A male-linked translocation has been used by Laven et al., (1971) in a genetic control program for *Culex pipiens*. However, a disadvantage of Y-linked translocations is that the genetic load introduced in a population is only temporary. Due to semi-sterility, translocation heterozygotes will rapidly disappear from the population, unless there is a compensation by an enhanced mating competitiveness of the heterozygotes (Curtis & Hill, 1971; Curtis, 1975).

In recent years there has been an increasing interest in male-linked translocations because these can be used for genetic sexing methods (Baker et al., Curtis, 1978 and Seawright et al., 1978). For the reduction of mass rearing costs of the sterile insect release methods (SIRM) or to exclude the release of biting females it is important to be able to produce only males. In the onion fly a large field experiment (25 ha.) has been carried out (Loosjes, M., pers. comm. and Loosjes, 1976) and a successful result has been obtained following the release of sterilized flies. From an economic point of view a genetic sexing method would be very important for the mass rearing of the onion fly.

More than 80 translocations have been isolated and many translocations have been analysed cytologically. Only three of these are sex-linked, two X- and one Y-autosome translocation. This low number is due to the relative small size of the sex-chromosomes, each about 4% of the total chromosomal length. One X-linked translocation has been studied extensively by van Heemert (1974).

Two types of sex-determination, XX (♀) / XY_1 (♂) and XX (♀) / XXY_2 (♂) coexist in *H. antiqua* (Vosselman, 1978). The male-linked translocation T(Y_1-2) between chromosome 2 and the acrocentric Y_1 was studied and the results about breakpoint positions, meiotic disjunction and fertility are reported. The notation Y instead of Y_1, will be used from now on. In a following article (Vosselman & van Heemert, 1980) results will be presented on the disjunction in males heterozygous for two translocations obtained from crosses between T(Y-2) males and females homozygous for an autosomal translocation T(2-6).

Material and method

Adults, larvae and pupae were reared at 23°C, approximately 70% r.h. in an incubator and sterility was assessed by classifying eggs as white (unfertilized), empty (hatched larvae) or brown (late embryonic lethals) using a stereomicroscope (12x). The sterility was defined as the percentage of brown eggs after subtraction of the white eggs from the total number of eggs (further details see van Heemert, 1974).

Seven days old males were irradiated with 2 Gy (0.2 krad) fast neutrons and F_1 males and females were testcrossed with control females. Those matings showing fertility reduced to 80% or less were retained and in the progeny of one F_1 male showing a very low egg hatch of 30% a Y-autosome translocation

T(Y-2) was identified. Testes of one-day-old males were dissected in Levy's saline solution and subsequently put in water for 5 minutes before being stained in lacto-acetic orcein (LAO). Squashing was carried out in 45% acetic acid and cytological analysis could be carried out immediately. Eggs were placed into a drop of LAO (2%) and dechorionated with a pair of fine needles. The vitelline membrane was ruptured and the embryonic tissue was stained at least one hour before squashing in acetic acid. Photographs were made from temporary preparations with a Zeiss photomicroscope using a high contrast Agfa-Gevaert ortho negative film (12 DIN).

Results and discussion

It was concluded from the strictly holandric inheritance of T(Y-2) that Y and not the X chromosome was involved. This could not be established cytologically due to the morphological correspondence between the two. In contrast to autosomal and X-autosome translocations, Y-autosome translocations can be easily maintained without the use of markers, cytological analysis or fertility screening. Since males are achiasmatic no recombination occurs, thus a Y-linked translocation cannot be converted into an X-linked one. Figure 1 shows the chromosomes of a normal and a translocated karyotype. The intimate meiotic pairing and the similarity in length between the X-chromosome and the short arm of chromosome 2^Y and similarly for chromosome Y^2 and the short arm of chromosome 2 indicate that the breakpoints in both chromosomes are located in the vicinity of the centromeres (Fig. 1d). On the contrary the meiotic pairing between Y^2 and X is loose or absent because the only homologous parts of chromosome X and Y^2 are the centromeric regions. Data on somatic pairing confirmed the very little or even absent affinity between Y^2 and X. In over one hundred somatic metaphase cells which were analyzed, mostly Y^2 and X were not associated.

Data from a 'simple' X-linked translocation (van Heemert, 1974) having a segment of chromosome 3 attached terminally (or almost so) to the X-chromosome, indicated that affinity between the translocated sex-chromosome X^3 and either the normal X- or Y-chromosome was very strong in both mitotic and meiotic stages. This was apparently due to the fact that these chromosomes have both centromeric and telomeric segments in common (van Heemert, 1977).

It will be shown elsewhere (Vosselman & van Heemert, 1980) that the male determining factor(s) has (have) been translocated to chromosome 2^Y, which is in accordance to the observation that the greater part of the Y-chromosome has been translocated.

As mentioned before the fertility in the first generation following irradiation was only 30%. In later generations the fertility increased to the control level (± 95%). Most probably this discrepancy can be attributed to the presence of an additional rearrangement and/or dominant lethality factors in the first generation and apparently disappeared subsequently.

In Table 1 data on meiotic disjunction of the translocation are presented. Since the four chromosomes of the translocation complex are easy recognisable a M II analysis could be compared with embryonic karyotype analysis. A high percentage (93.6) of alternate (2X and $2^Y Y^2$) gametes were scored in M II cells. Embryos in eggs produced by females which had mated to T(Y-2) males gave a somewhat lower percentage (86.6), but this percentage was not significantly different from the percentage as scored in M II cells (test on heterogeneity, $\chi^2_{[1]} = 3.8$, $0.05 < p < 0.10$). The average percentage (90%) of

Fig. 1. *Hylemya antiqua:* (a) normal karyotype, spermatogonial metaphase (bar represents 5 μm); – (b) spermatogonial metaphase from a T(Y-2) male (incomplete cell, arrows point to centromeres); – (c) translocation complex in a T(Y-2) male in two diakinesis-prometaphase cells (arrows point to centromeres); – (d) diagram of translocation heterozygote, with breakpoint positions.

Table 1

Segregation (in %) in M II cells in T(Y-2) males and in eggs and larvae of normal females testcrossed with T(Y-2) males in *H. antiqua*. (nnd = numerical non-disjunction)

	number	Alternate 2X	$2^Y Y^2$	total	Adjacent-1 $2^Y X$	$2Y^2$	Adjacent 2 Y^2X	$2\,2^Y$	nnd-1 $2^Y Y^2 X$	2	nnd-2 $2Y^2X$	2^Y
M II	125	53.6	40.0	93.6	0.8	1.6	–	–	0.8	2.4	–	0.8
Embryos	139	42.5	44.1	86.6	4.7	3.8	1.1	–	–	–	3.2	0.6
Larvae (yong)	55	40.0	50.9	90.9	–	–	–	–	5.5	–	3.6	–

the alternate orientations (M II and eggs) is slightly lower than the egg hatch percentage of about 95% and this difference might be attributed to the survival of some unbalanced karyotypes into the larval stage. This applies to the karyotypes $2^Y Y^2 X$, $2Y^2 X$ (Table 1) and $2Y^2$ as will be demonstrated elsewhere (Vosselman & van Heemert, 1980).

Since the only homologous chromosome parts of the acrocentric X and Y^2 are the centromeric regions, chain quadrivalents with X and Y^2 in terminal position are formed at MI. With an intact chain quadrivalent orientation types adjacent -1 and numerical non-disjunction -1 and -2 are supposed to be unstable. As shown in Fig. 2 either X or Y^2, or both X and Y^2 lack adjacent centromere(s) orientating to the opposite pole. Consequently a balancing counter-force for these chromosomes, which is important for the stability of the orientation, is lacking (Sybenga, 1975). It is suggested, therefore, that in these cases a reorientation will occur resulting in high frequencies of the stable alternate orientation. Probably due to incomplete reorientation or by an earlier disjoining of X and/or Y^2 from the multivalent followed by reorientation (Vosselman & van Heemert, 1980) adjacent -1 and numerical non-disjunction -1 and -2 can occur in low frequencies. Also adjacent -2 was observed in a very low frequency (Tab. 1). Perhaps this orientation type is unstable as a consequence of 'the unbalanced position of the chromosomes in respect to the equator (the two long chromosomes 2 and 2^Y are directed to one pole and the two small chromosomes X and Y^2 to the other). However, other factors cannot be excluded, as in six other translocations (van Heemert, 1974 and Vosselman, pers. comm.) adjacent-2 was observed also very infrequently when males were the translocation carriers.

As shown in Table 1 numerical non-disjunction occurred rather seldom. Ullerich (1963) had observed a low (2%) numerical non-disjunction frequency as well in a Y-linked translocation in *Phormia regina* in a similar Y-linked translocation as discussed here. The same was established in translocation males of the X-linked translocation (van Heemert, 1974).

As indicated earlier Y-linked translocations can be exploited for the development of a genetic sexing technique. The aim of such a technique is preferentially killing the females in a young larval stage to reduce mass rearing costs. This method using insecticides has already been successfully developed in some mosquito species (Curtis, 1978 and Seawright et

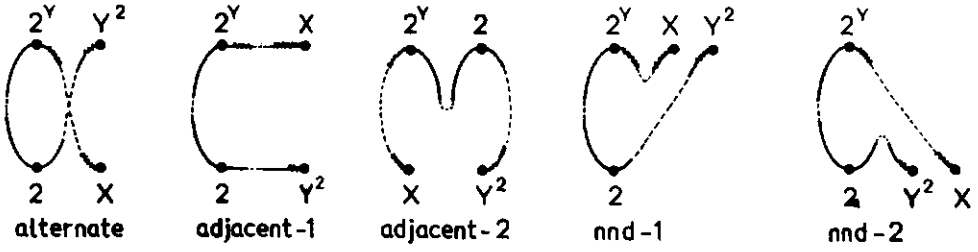

Fig. 2. Quadrivalent shapes as expected in T(Y-2) male meiosis (nnd = numerical non-disjunction).

al., 1978). Other types of conditional lethal systems such as temperature sensitive lethals (Baker et al., 1978) can be used as well.

The Y-linked translocation as studied here has been crossed with dieldrin resistant females for two generations to obtain dieldrin resistant males. It appeared that the locus for dieldrin resistance was not on chromosome 2 or Y. Therefore it was decided to re-irradiate dieldrin-resistant T(Y-2) males to obtain an additional translocation between the chromosome carrying the resistant allele (R) and 2^Y. By giving a discriminative dose of dieldrin (2 μg) to flies from crosses of males heterozygous for the dieldrin gene (Rr) and susceptible females (rr), the susceptible ones will die and the semi-dominant flies will survive. In case an additional translocation has been induced between the chromosome carrying the dieldrin resistant allele R and 2^Y all males will survive and all females will die.

Rabbani & Kitzmiller (1976) suggested the use of a Y-linked translocation in *Anopheles albimanus* for testing sexual competitiveness and female monogamy, instead of using morphological body markers with the risk of lower fitness. In cage experiments followed by cytological analysis this test mechanism was used succesfully.

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MEIOTIC DISJUNCTION AND EMBRYONIC LETHALITY IN SEX-LINKED DOUBLE-TRANSLOCATION HETEROZYGOUS MALES OF THE ONION FLY, *HYLEMYA ANTIQUA* (MEIGEN)

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SUMMARY

The frequencies of disjunction types in double-translocation heterozygous males ($2^{6}2^{Y}6^{2}6XY^{2}$) in *Hylemya antiqua* have been established in MII cells and eggs of testcrosses.

Several disjunction types occurred but four predominated. A correlation was found between the frequencies of the disjunction types and the relative position of the centromeres. The frequency of numerical non-disjunction (NND) was 4%. Differences in frequency of NND between sex-linked and autosomal translocations of *H. antiqua* are discussed. A good correspondence between the frequencies of unbalanced karyotypes, and embryonic and larval mortality was found. The total genetic load which can be induced by the T14/T61 males is estimated to be 60-65%. Some duplication/deficiency karyotypes appeared to be viable in pupal and even adult stages. In $2^{6}2^{6}2^{Y}6^{2}6^{2}X$ males a regular coorientation between 2^{Y} and X was observed, in spite of non-homologous centromeres and a complicated synapsis of 2^{Y}. Application possibilities of the present material for genetic control of *H. antiqua* are discussed.

Keywords: *Hylemya antiqua*, double-translocation heterozygotes, disjunction types, genetic control.

INTRODUCTION

In several insect species double-translocation heterozygotes have been produced for genetic insect control purposes (reviewed by Robinson, 1976; Cochran and Ross, 1977; Petersen et al., 1977; Suguna et al., 1977; Terwedow et al., 1977). Attention has been mainly paid to the genetic load which can be induced. However, the current knowledge about meiotic disjunction of such complex translocation multivalents is limited. Difficulties in discriminating the separate chromosomes and inaccessibility of some insect species for cytogenetic investigations are the main reasons. In the onion fly *(Hylemya antiqua)* the frequencies of the disjunction types of translocation multivalents can be estimated by metaphase II classifications or by analysing eggs (embryos) of testcrosses (van Heemert, 1974 a, b). An advantage of eggs is, that somatic pairing makes it possible to discriminate, in many cases, between translocated and standard chromosomes of about equal length.

The present paper deals with the segregation types of double-translocation heterozygous T14/T61 males. These males were obtained by crossing females homozygous for an autosomal translocation (T14/T14) with males heterozygous for a Y-linked translocation (T61/+). The normal chromosomal complement of *H. antiqua* comprises 5 autosomal pairs and sex determination is either XX(♀)/XY_1(♂) or XX(♀)/XXY_2(♂) (Vosselman, 1978). The breakpoints of the Y-linked translocation T61 are located in the short arm of autosome 2 and in the long arm of the acrocentric Y_1 chromosome(Fig.1)both in the vicinity of the centromere (van Heemert and Vosselman, 1980). Chromosome Y_1 will be further indicated as Y and the translocated Y_1 chromosome as Y^2. In MI cells of the T61/+ males a chain-of-four with X and Y^2 in terminal positions was generally observed. This was attributed to an earlier disjoining of the centromeric regions of X and Y^2. Since the segregation of the translocation multivalent was predominately alternate, the fertility of the T61/+ translocation heterozygotes was hardly reduced. Translocation T14 concerns a rearrangement between the long arms of the autosomes 2 and 6, with proximally located breakpoints. The orientation of the translocation multivalent (ring quadrivalents) in T14/+ heterozygous males was alternate or adjacent I, in a ratio of 7:3. Other orientations were not observed. Individuals homozygous for translocation T14 showed normal fertility (Vosselman, 1980).

Recently Baker et al. (1978), Curtis (1978) and Seawright et al. (1978) have emphasized the significance of genetic sexing systems for genetic insect control. Also in *H. antiqua* it will be attempted to develop such a sexing system in order to reduce costs of mass rearing for the sterile insect release method (van Heemert and Vosselman, 1980). In this report it will be demonstrated, that the double-translocation heterozygous males T14/T61 are potentially suitable for the development of a genetic sexing system.

MATERIALS AND METHODS

Translocations T14 and T61 have previously been induced in sperm by irradiation with X-rays and fast neutrons respectively, in both cases with a dose of 2 Gy (200 rad). By crossing T14-homozygous females ($2^6 2^6 6^2 6^2 XX$) with T61-heterozygous males ($2\ 2^Y\ 6\ 6XY^2$) a male offspring consisting of exclusively double-heterozygotes T14/T61 ($2^6 2^Y 6^2 6\ XY^2$) and some duplication/deficiency karyotypes were obtained. Since in both translocations chromosome 2 is involved and males are achiasmate, the only balanced gametes which are formed by the double-heterozygotes are $2^6 6^2$ X and $2^Y\ 6\ Y^2$. Consequently a "pure breeding" stock of T14-homozygous females and T14/T61 double-heterozygous males could be produced by crossing the double-heterozygous males with the T14-homozygous females. The flies with duplication/deficiency karyotypes which occurred did not reproduce. The rearing methods and the cytological techniques used for larvae and adults were reported earlier (Vosselman, 1978). Egg-hatch reduction was determined after incubation of the eggs at 25 ^{o}C during three days. The dead embryos could be recognized by a brown colour. Unfertilized eggs (white colour) have been excluded from the egg-hatch calculations. For the karyotype analysis of embryos, eggs of 14-20 hours were used after incubation at 20 ^{o}C. After careful removing of the chorion and the vitelline membrane in a drop of 2% lacto acetic orcein (LAO), the embryo was divided into small pieces, stained for 15-45 minutes in this drop and subsequently squashed in 45% acetic acid. Photographs were made with a Zeiss photomicroscope on an Agfa-Ortho 25 professional film (12 DIN).

Table 1. Karyotype frequencies in MII cells and in eggs of testcrosses of double-heterozygous $2^6 2^Y 6^2 6\ XY^2$ males of *H. antiqua*. The male-derived chromosomes are only given . A discrimination between 6 and 6^2 was not always possible.

Eggs							MII	Total (MII + eggs)	
chr.	chr. 6^2	disj. type	chr. 6	disj. type	chr. 6^2 or 6	total	chr. 6^2 or 6	number	%
2^6 X	22*	Ia	5	Ib +	5	32	66	98	26.6
2^Y Y^2	7	Ib	20*	Ia	6	33	57	90	24.5
2^6 2^Y	15*	IIa	10*	IIb	5	30	51	81	22.0
X Y^2	9	IIb	6	IIa	3	18	55	73	19.8
2^Y X	3	IIIa	0	IIIb	0	3	3	6	1.6
2^6 Y^2	0**	IIIb	0	IIIa	0	0	5	5	1.4
2^6 X Y^2	1*		0		0	1	2	3	0.8
2^Y	0		0		0	0	6	6	1.6
2^6 2^Y Y^2	0**		0**		0	0	1	1	0.3
X	0		1		0	1	2	3	0.8
2^6 2^Y X	0**		0**		0	0	2	2	0.5
Y^2	0		0		0	0	0	0	0.0
Total	57		42		19	118	250	368	99.9

\+ Code for disjunction types of Fig. 3

* Viable in larval and adult stage

** Probably viable in larval and adult stage, but not observed in these stages

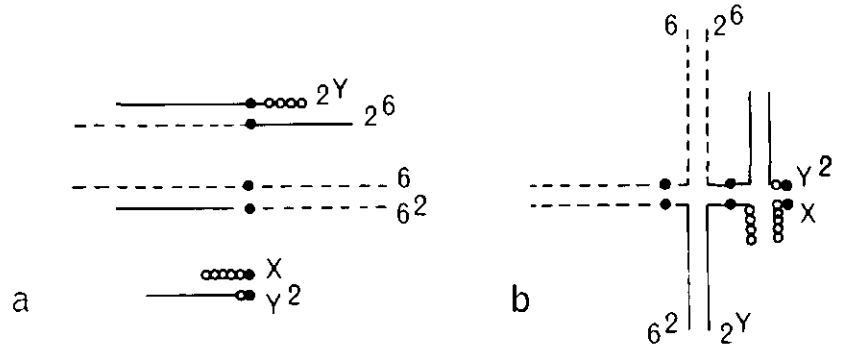

Fig. 1. a - Chromosomes involed in translocation complex of double-heterozygous $2^6 2^Y 6^2$ 6 X Y^2 males. b - Pairing-configuration, diagrammatically.

RESULTS

Disjunction types of the double-heterozygotes.

In Fig. 1 the chromosomes involved in the translocation complex of the double-heterozygous males are diagrammatically represented. Fig. 2A shows a spermatogonial metaphase. In the first meiotic metaphase a chain-of-six with X and Y^2 in terminal positions (Fig. 2B) is generally observed and occassionally a chain-of-five and an univalent (Fig. 2C).

In metaphase II cells of the double-heterozygous $2^6 2^Y 6^2$ 6 XY^2 males it was often not certain whether chromosome 6^2 or 6 was present, therefore no discrimination was made for these chromosomes in Table 1. The other four chromosomes involved in the translocation complex could easily be discriminated. Eggs (young embryos) were in general better classifiable, due to the presence of somatic pairing and because many cells per embryo could be scored. However, not always a discrimination between 6^2 and 6 was possible, although better than in MII cells (Table 1). About 50% of the MII cells and embryos correspond to the disjunction types Ia and Ib (Table 1 and Fig. 3). Disjunction type Ia, producing the balanced gametes $2^6 6^2$ X and 2^Y 6 Y^2, was the most frequently occurring one. The ratio of Ia to Ib in eggs was 42 (22+20) : 12 (7+5) (Table 1) and in MII cells, as far as a discrimination between 6 and 6^2 was possible, about 2 : 1 to 3 : 1 (not indicated in Table 1).

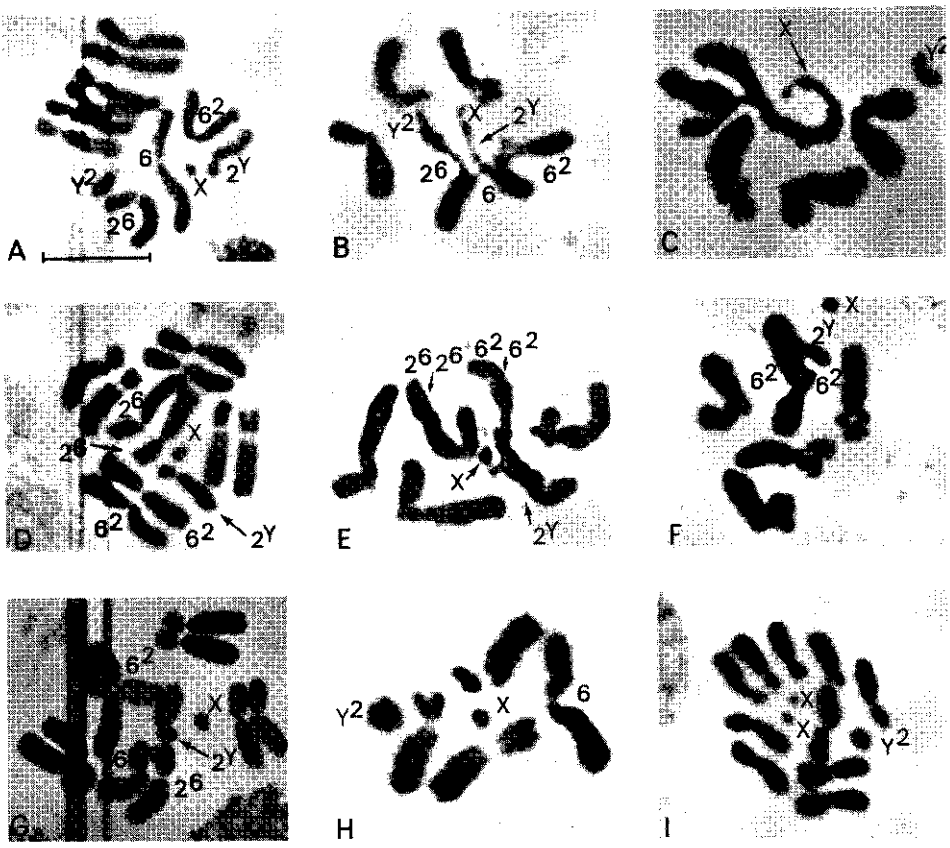

Fig. 2. Karyotypes of *H. antiqua*. A-C: double-heterozygous $2^6 2^Y 6^2$ 6 X Y^2 males. A - spermatogonial metaphase; B - prometaphase/metaphase I; C - prometaphase/metaphase I, Y^2 apart from multivalent; D-F: duplication/deficiency $2^6 2^6 2^Y 6^2 6^2$ X males; D - spermatogonial metaphase; E - prometaphase/metaphase I*; F - prometaphase/metaphase I, X apart from multivalent*; G - spermatogonial metaphase of duplication/deficiency $2^6 2^6 2^Y 6^2$ 6 X male; H - M II cell of male, 6 X Y^2; I - spermatogonial metaphase of duplication/deficiency $2^6 2^6 6^2 6^2$ X X Y^2 female. Bar represents 10 µm.

*E,F: note normal double pairing in 2^6 and 6^2 but triple pairing in 2^Y, cf. Fig. 4.

Disjunction types IIa and IIb, corresponding to 41.8% of the MII cells and eggs scored, seem to occur in about equal frequencies. Types IIIa and IIIb were found in a frequency of 3%, but probably IIIa does not occur at all. All six disjunction types (Ia to IIIb) are the consequence of a 3 - 3 segregation and a coorientation between 6^2 and 6 and included together 96% of the MII's and eggs. The remaining 4% includes all cases of a 2 - 4 segregation, but with a coorientation between 6 and 6^2 as well.

Viability of unbalanced karyotypes and disjunction in duplication/deficiency $2^6 2^6 2^Y 6^2 6^2 X$ *males.*

Not all unbalanced karyotypes died in the embryonic stage and some of these even reached the adult stage (Table 2). The duplication/deficiency karyotypes $2^6 2^6 2^Y 6^2 6^2$ X (Figs. 2D-F) and $2^6 2^6 2^Y 6^2$ 6 X (Fig. 2G) corresponding

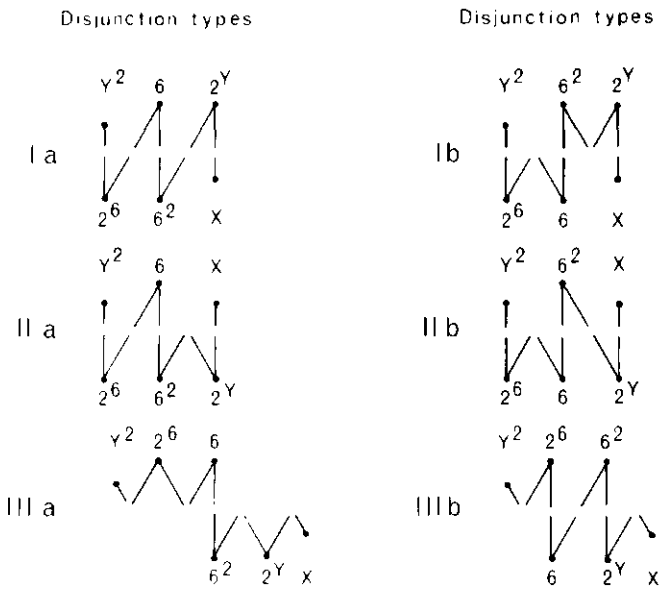

Fig. 3. Disjunction types of the chain-hexavalent of double-heterozygous $2^6 2^Y 6^2$ 6 X Y^2 males of *H. antiqua* assuming coorientation between 6^2 and 6 and excluding numerical non-disjunction (see text).

to the gametes $2^{6}2^{Y}6^{2}$ and $2^{6}2^{Y}$ 6 (Table 2), both have a male phenotype. Because the translocation breakpoint in chromosome Y is in the vicinity of the centromere, the greatest part of Y is present in these individuals. The duplicated chromosome segments in $2^{6}2^{6}2^{Y}6^{2}6^{2}$ X are the long arm and centromeric region of 2 (Fig. 4) and in $2^{6}2^{6}2^{Y}6^{2}$ 6 X almost the entire long arm of 6 and the centromeric region of 2. From Tables 1 and 2 it can be derived that a significant decrease in the relative frequency of these two duplication/deficiency karyotypes from embryonic to adult stage existed. This has to be attributed to a lower viability in larval and pupal stage, manifested also by a very small body size. Indications were obtained that the viability of $2^{6}2^{6}2^{Y}6^{2}$ 6 X individuals was more reduced than of $2^{6}2^{6}2^{Y}6^{2}6^{2}$ X. Another duplication/deficiency karyotype $2^{6}2^{6}6^{2}6^{2}$ XXY^{2} (Fig. 2I) appeared to be viable in the adult stage; its phenotype was female.

In six $2^{6}2^{6}2^{Y}6^{2}6^{2}$ X males metaphase II cells could be analysed (Table 3). In spite of non-homologous centromeres an almost perfect coorientation between 2^{Y} and X was found.

Fertility reduction of the double-heterozygotes.

The percentage of dead embryos (brown eggs) in testcrosses of the double-heterozygous males was 39.5 (3846 eggs, unfertilized eggs excluded). This corresponds rather well with the data from the cytological analyses of MII cells and eggs.The majority of embryonic mortality is caused by the gametes: 6^{2} XY^{2} and 6 XY^{2} (half of the gametes from disjunction types IIa and b), 2^{6} 6 X and $2^{Y}6^{2}Y^{2}$ (disjunction type Ib). The frequencies of these gametes can be derived from the combined data of MII cells and eggs (Table 1). Assuming the ratio of the disjunction type Ia to Ib to be about 3 : 1 (see above), the total frequency of these four gametes is estimated to be 34% ($\frac{1}{2}$ x 41.8 + $\frac{1}{4}$ x 51.1). Other karyotypes dying in embryonic stage, all occurred in low numbers and the total frequency of these is estimated to be 3-5% (Table 1). The total genetic load induced by the double-heterozygous males is 20-25% higher, because the majority of $2^{6}2^{6}2^{Y}6^{2}6^{2}$ X and $2^{6}2^{6}2^{Y}6^{2}$ 6 X individuals and some other unbalanced karyotypes died either as larvae or as pupae; the surviving ones dit not reproduce.

Table 2. Karyotype frequencies (larvae and adults) in testcrosses of double-heterozygous $2^6 2^Y 6^2 6$ X Y^2 males of *H. antiqua*. The male-derived chromosomes are only given. In larvae a discrimination between 6 and 6^2 was not always possible. Cf. Table 1.

	Larvae							Adults			
chr.	chr. 6^2	disj. type	chr. 6	disj. type	chr. 6^2 or 6	total	%	chr. 6^2	chr. 6	total	%
2^6 X	47	Ia*	†	Ib*	-	47	40.9	44	†	44	37.9
2^Y Y^2	†	Ib	41	Ia	-	41	35.7	†	60	60	51.7
2^6 2^Y	15	IIa	7	IIb	4	26	22.6	8	3	11	9.5
X Y^2	†	IIb	†	IIa	-	-	-	†	†	-	-
2^6 X Y^2	1		†		-	1	0.9	1	†	1	0.9
Total	63		48		4	115	100.1	53	63	116	100.0

* Code for disjunction types of Fig. 3

† Lethal in late embryonic stages

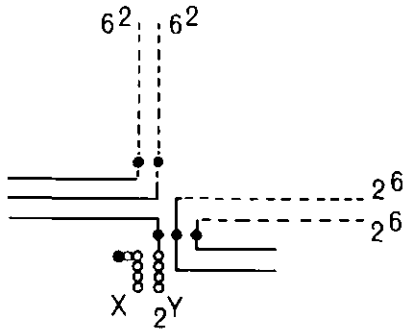

Fig. 4. Pairing-configuration, diagrammatically, in duplication/deficiency $2^6 2^Y 6^2 6^2$ X males.

Table 3. MII scores in duplication/deficiency $2^6 2^6 2^Y 6^2 6^2$ X males.

MII type	Number
$2^6 6^2 2^Y$	103
$2^6 6^2$ X	99
$2^6 6^2$	2
$2^6 6^2$ X 2^Y	1

DISCUSSION

Disjunction types of the double-heterozygotes.

From the MII and egg scores (Table 1) it can be concluded that mainly a 3 - 3 segregation occurred and that the chromosomes 6 and 6^2 always co-oriented. In that case six different disjunction types can be distinguished (Fig. 3). Sybenga (1975) has emphasized that the position of a centromere in respect to its neighbours is an important factor for its stability (probability for reorientation). Also in the present case a correlation exists between the frequencies of the six disjunction types given in Fig. 3 and the relative positions of the centromeres. The position of a centromere in respect to its neighbours is indicated with *cis* when oriented to the same pole and with *trans* when oriented to the other pole. Disjunction types IIIa and IIIb, observed together in a frequency of only 3%, have to be considered as unstable because in IIIa X, Y^2, 2^6 and 2^Y and in IIIb X and Y^2 do not have a neighbouring centromere in trans-position (no counterforce for the pull exerted by the spindle fibres). In the remaining four disjunction types X and Y^2 always have a trans-position in respect to their(single) neighbour. The other four centromeres have two neighbours and are positioned as follows: in Ia all in trans-trans, in IIa and IIb two in trans-trans and two in cis-trans and in Ib all in cis-trans. Assuming that a trans-trans position is more stable than cis-trans, it may be expected that Ia was the most and Ib the least frequently observed disjunction type and that IIa and IIb occurred in intermediate frequencies.

The frequency of numerical non-disjunction (NND) among the 250 MII cells and 118 eggs analysed amounted to 4%, about the same value as was found in 2 2^Y 6 6 X Y^2 (T61/+) males (van Heemert and Vosselman, 1980). For two X-linked translocations, one between X and 3 (van Heemert, 1974a) and one between X and 4 (Vosselman, unpublished), the level of NND scored in heterozygous males was 2% and 15% respectively. In contrast, in males heterozygous for autosomal translocations NND has very rarely been observed, although five different translocations including T14 were studied (Vosselman, in prep.). In females, which in contrast to males are chiasmate, slightly different results were found which will be discussed elsewhere. It is suggested that the difference in NND between autosomal and sex-linked translocations scored in males is a consequence of an earlier disjoining of the sex chromosomes (translocated or non-translocated) from the multi-

valent. When this is followed by reorientation of either the multivalent or the univalent, NND can be the result. The early separation of the sex chromosomes from the multivalent can probably be attributed to the acrocentric (telocentric) nature of the sex chromosomes; the connection with the multivalent rests on only one short chromosome arm. In contrast, all autosomes have two arms bound (Fig. 2).

In *Culex pipiens* high frequencies of NND were reported by Jost and Laven (1971) for translocations involving chromsome 1. Lack of chiasmata and earlier terminalisation of chiasmata in chromosome 1 was considered to be the reason. For T70H/+ males in the mouse de Boer (1976) found high numbers (34-50%) of trivalent plus univalent configurations, but only 4-9% of the MII cells were aneuploid. Non-random segregation of the univalent was given as explanation for this discrepancy.

Viability of unbalanced karyotypes and disjunction in duplication/deficiency $2^6 2^6 2^Y 6^2 6^2 X$ *males.*

In *H. antiqua* duplication/deficiency karyotypes produced by translocations are mostly lethal in the embryonic stage, but some can survive to the larval stage (Robinson and van Heemert, 1975).

Individuals with a duplication for half the long arm of chromosome 3 showed a reduced viability but some stayed alive into the adult stage (van Heemert, 1974a, b). On the contrary sex chromosome aneuploidy has in general no impact on the viability of the adults (van Heemert, 1974a, b; Vosselman, 1978). In the present case all three duplication/deficiency karyotypes $2^6 2^6 2^Y 6^2 6^2$ X, $2^6 2^6 2^Y 6^2$ 6 X and $2^6 2^6 6^2 6^2$ XXY^2 which were (partially) viable in the adult stage, had only a deficiency for a part of the Y-chromosome. The duplicated segments concerned different chromosomes. In *Aedes aegypti* (Ved Brat, 1974) and in *Glossina austeni* (Curtis et al., 1972) viable duplication/deficiency karyotypes have been reported as well.

As $2^6 2^6 2^Y 6^2 6^2$ X and $2^6 2^6 2^Y 6^2$ 6 X were males and $2^6 2^6 6^2 6^2$ XXY^2 was a female, it is obvious that the male determining gene(s) is (are) located on the large segment of the Y-chromosome, translocated to the 2^Y-chromosome.

An interesting observation was the regular coorientation between 2^Y and X in $2^6 2^6 2^Y 6^2 6^2$ X males (Table 3). As is shown in Fig. 4, the greatest part of the long arm of 2^Y is homologous with 6^2, most of the short arm with X

and the centromeric region with 2^6. Considering the rather complicated synapsis of 2^Y (Fig. 2 E), at first sight the regular coorientation between 2^Y and X was surprising. However, on further consideration there seems to be a reasonable explanation, assuming firstly that 2^6 and 6^2 will both preferentially orient on their fully homologous pairing partners and secondly, that the most stable orientation will be achieved when 2^Y and X coorientate. In that case for both 2^Y and X there is a counterbalance to the force of the pulling spindle fibres, which is essential for a stable orientation (cf. Sybenga, 1975). It is apparent that homology of centromeres is not necessary for a regular coorientation, as has also been suggested e.g. by John and Lewis (1965) and Douglas (1968).

Application possibilities for genetic control

A pure breeding stock which is permanently reduced in fertility can be obtained by crossing T14-homozygous ($2^6 2^6 6^2 6^2$ XX) females with double-heterozygous ($2^6 2^Y 6^2$ 6 XY^2) males. However, the applicability of this for genetic insect control is limited, as it seems impossible to replace a target population completely by these karyotypes. When this condition is not fulfilled or when immigration occurs, it is inevitable that the double-heterozygous males, as a consequence of their fertility reduction, will disappear from the population.

Conditional lethality, when determined by a locus on one of the chromosomes involved in a Y-autosome translocation, can be exploited for genetic sexing (elimination of females). Since in a stock consisting of T14-homozygous ($2^6 2^6 6^2 6^2$ XX) females and T14/T61 double-heterozygous ($2^6 2^Y 6^2$ 6 XY^2) males, chromosome 6 is also inherited in a strictly holandric way and in males recombination is absent, loci on this chromosome 6 as well as on 2^Y and Y^2 can be used for this purpose. This is of interest because a locus for alcohol dehydrogenase has been localized on chromosome 6 (van Heemert and Witteveen-Pillen, 1980). When in *H. antiqua* a suitable alcohol dehydrogenase-null mutant can be induced, it must be possible to produce a pure breeding stock of alcohol-positive (Adh^+ Adh^o) T14/T61 males and susceptible (Adh^o Adh^o) T14-homozygous females. In *Drosophila melanogaster* several alcohol dehydrogenase-null mutants could be induced (cf. Gerace and Sofer, 1972) and Robinson and van Heemert (1980, pers.comm.) have recently developed a genetic sexing system in this species involving such a mutant.

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MEIOTIC SEGREGATION OF FIVE DIFFERENT RECIPROCAL TRANSLOCATIONS IN THE ONION FLY, *HYLEMYA ANTIQUA* (MEIGEN)

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ABSTRACT

For one translocation (T14) with short interstitial segments in *Hylemya antiqua* significant differences in segregation behaviour between males and females were observed. In males the ratio of alternate : adjacent 1 : adjacent 2 was approximately 7:3:0 and in females about 8:1:3. This difference is attributed to the difference in type of chromosome association. Female meiosis is chiasmate and male meiosis is achiasmate. It is suggested that meiotic pairing in males results in relative short "Coorientation Determing Distances" (CDD's) between homologous centromeres which favours alternate and adjacent 1 segregation. In females because of non-localized chiasmata on the average no differences in CDD between homologous and non-homologous centromeres are expected. This might explain the occurrence of coorientation between non-homologous centromeres resulting in adjacent 2 segregations. Four other translocations with longer interstitial segments than T14 showed in males as well as females predominantly an alternate and adjacent 1 segregation, adjacent 2 was hardly found (0-3.6%). The longer distance between non-homologous centromeres is probably the reason.

INTRODUCTION

Individuals heterozygous for a single reciprocal translocation may show four different types of orientation in the first meiotic division giving numerically equal segregation: alternate 1 and 2, and adjacent 1 and 2 (John and Lewis, 1965; Sybenga, 1975). Alternate 1 and 2 are usually assumed to be indistinguishable but according to Endrizzi (1974), Cochran (1976, 1977) and Lacadena and Candela (1977) under certain conditions it would be possible to discriminate between the two types of alternate orientation. With sufficient variation in chromosome characters to recognize specific chromosomes it is possible to distinguish the two types of adjacent orientation: adjacent 1 and 2. In the former non-homologous and in the latter homologous centromeres pass to the same pole. With interstitial chiasmata alternate and adjacent 1 become

identical (cf. Burnham, 1956). In *Hylemya antiqua* this can only occur in females, since in males chiasmata are normally absent.

Burnham (1950, 1956) has made the significant observation that in ring-forming maize translocations with one or two long interstitial segments adjacent 2 segregations are rare or absent. On the contrary, translocations with short interstitial segments showed on an average a ratio of alt.:adj.1 : adj.2 of 2:1:1. In rye, by inbreeding and selecting lines with high and low alternate frequencies, Sun and Rees (1967) established a clearly negative correlation between chiasma frequency and alternate disjunction not considering interstitial chiasmata. Since the most pronounced difference was found between quadrivalents with four and five chiasmata respectively, Sybenga (1975) attributes the bias towards adjacent segregation in the latter type to the presence of proximal chiasmata. Such a proximal chiasma may result in a more rigid structure, which may favour an adjacent segregation (Darlington and Gairdner, 1937). For T70H translocation heterozygotes of *Mus musculus*, with acrocentric chromosomes, de Boer (1980) suggests a causal relationship between the presence of a proximal interstitial chiasma in late meiotic cells and the occurrence of adjacent 2. In *Hylemya antiqua* there is a good opportunity to study indirectly possible effects of chiasmata on the segregation behaviour of quadrivalents, since crossing-over is restricted to the female sex. One of the translocations (T14) reported here was used in cage experiments in order to establish the value of this translocation for genetic control of the onion fly (Robinson et al., 1980; Vosselman, 1980). As significant differences in segregation behaviour between the sexes were found, we have decided to study this and four more translocations in more detail.

MATERIALS AND METHODS

Translocation T14 (between chromosomes 2 and 6) was induced in sperm by irradiation with 2 Gy X-rays. The T14-heterozygotes (T14+) were maintained by repeated backcrosses to flies with standard chromosomes (++). By using an appropriate crossing scheme (Vosselman, 1980) a stock consisting of exclusively T14-homozygotes ($2^{6}2^{6}6^{2}6^{2}$) was obtained. Following irradiation of some T14-homozygous males with fast neutrons (1 Gy) and backcrossing with T14-homozygous females, the other four translocations were isolated. These translocations (DT_{1}, DT_{3}, DT_{8} and DT_{11}) were kept in the T14-homozygous background. Consequently, as far as meiotic behaviour is concerned, they can be considered as single reciprocal translocations.

For the MII-analysis squash preparations of testes from 1-2 d. old males were used. For further details see Vosselman (1978). The segregation in females and in some of the males was studied by karyotyping young embryos from test-crosses. For this purpose 14-20 h. old eggs were used. The chorion and vitelline membrane of these eggs were carefully removed in a drop of 2% lacto acetic orcein without previous fixation and the eggs were subsequently stained and fixed in the same medium for 20-40 minutes. Before squashing in 45% acetic acid the embryos were divided into several small pieces. Rearing conditions and methods were the same as in earlier studies (Vosselman, 1978). Embryonic lethality was determined by examining 3-4 d. old eggs kept at 25°C. Eggs with a white appearance and without other signs of embryonic development were considered as unfertilized. These eggs were excluded from the egg-hatch estimates. Since some duplication/deficiency karyotypes survived to the larval stage, also the pupation percentages (number of pupae divided by the total number of eggs except the unfertilized ones, multiplied by 100) were used as an indication for the ratio of balanced and unbalanced karyotypes. It has to be noted, however, that the larval survival is quite variable. In controls (crosses between T14-homozygotes or flies with standard chromosomes) the average pupation percentages were 80-85%. The embryonic lethality in these crosses amounted to 2-3%.

RESULTS

Translocation T14

The breakpoints of translocation T14 are located in close proximity to the centromeres, in the long arms of chromosomes 2 and 6 (Figs. 1 and 2). The translocated chromosome 2^6 has increased in length compared to 2 and is slightly longer than 3 and 4, while chromosome 2 is the smallest autosome. Chromosome 6^2 is smaller than 6 and is almost metacentric (Figs. 1 and 2).

In eggs from testcrosses of T14+ females (Table 1a) the karyotypes arisen from adjacent 2 or numerical nondisjunction (NND) could always easily be recognized (Fig. 2, f-h). From the 280 eggs classified in 11 test-crosses 25% corresponded to adjacent 2 and 2% to NND. Due to the small differences in length between 2 and 2^6 and between 6 and 6^2 it was much more difficult to discriminate between the karyotypes arisen from alternate and adjacent 1. After some slight modifications in the method of making preparations we were able to classify a sufficiently high percentage of eggs corresponding to

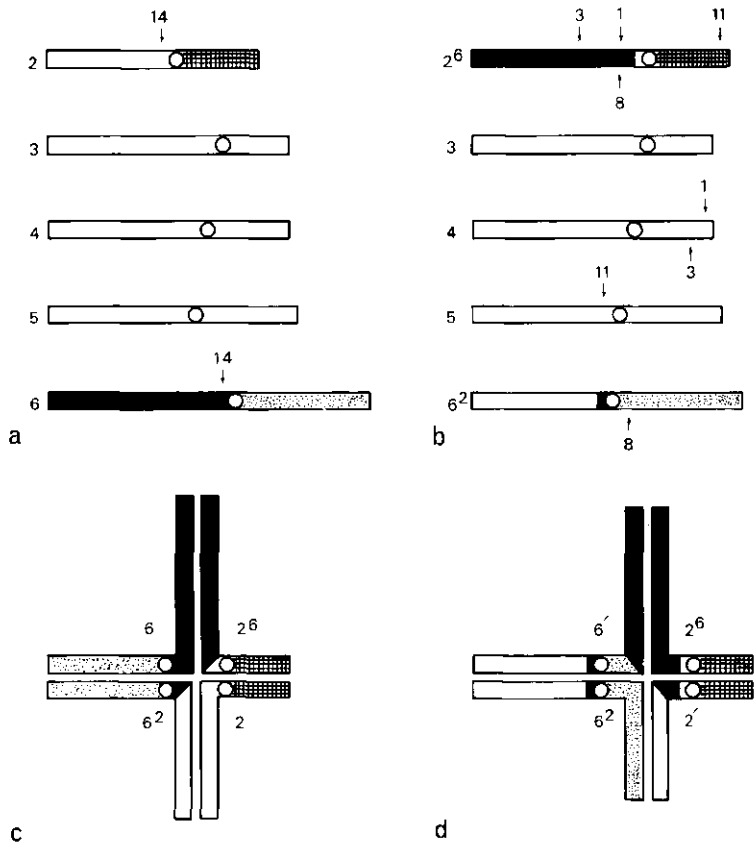

Fig. 1. a. Breakpoint positions of translocation T14 (note: the short and long arms of the chromosomes 2 and 6 are differently marked).
b. Breakpoint positions of translocations DT_1, DT_3, DT_8 and DT_{11}. These DT-translocations were induced in T14-homozygotes ($2^6 2^6 6^2 6^2$).
c. Quadrivalent of T14-heterozygote ($2^6 2 6^2 6$). d. Quadrivalent of DT_8-heterozygote ($2^6 2 6^2 6'$); $2'$ and $6'$ are the retranslocated chromosomes 2^6 and 6^2.

alternate and adjacent 1. This is the reason that for only two testcrosses of T14+ females the ratio of these segregation types are given (Table 1a). Five eggs could not be exactly karyotyped (either alternate or adjacent 1). When these five eggs are proportionally divided over both types, the percentages of alternate and adjacent 1 are equal to 61 (45/52 x 70.4) and 9 (7/52 x 70.4) respectively. Estimates of the frequencies of alternate and adjacent 1 can

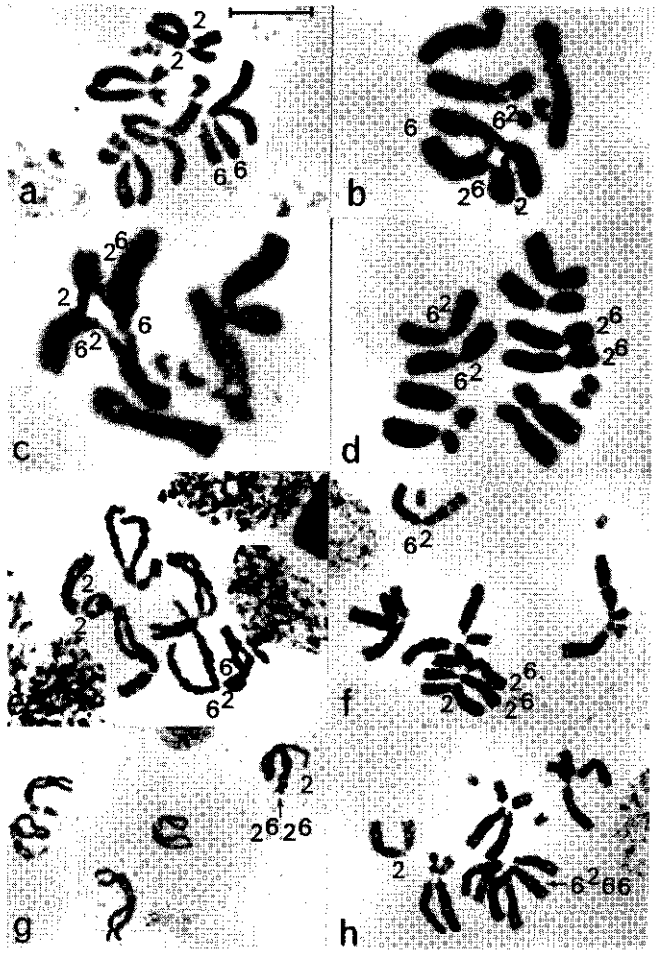

Fig. 2. a-h. Karyotypes of the onion fly. a. Normal karyotype (++), mitotic metaphase in larval ganglion cell. b. and c. Multivalent of heterozygous male ($2^{6}26^{2}6$), diakinesis/prometaphase I. d. T14-homozygous male ($2^{6}2^{6}6^{2}6^{2}$), spermatogonial metaphase. e-h. Duplication/deficiency karyotypes for translocation T14, mitotic metaphases in young embryos. Bar represents 10 µm.

Table 1a. Segregation types in females and males heterozygous for translocation T14 (2,6) (eggs of testcrosses of females and MII cells of males). A discrimination between alternate and adjacent 1 was not always possible (see text).

Disjunction type	Gametic or MII type	Females			Males
		n=2	n=9	Total(%)	n=10
Alternate	2 6, $2^6 6^2$	45	-	-	46
Adjacent 1	$2^6 6$, $2\ 6^2$	7	-	-	27
Alt. or adj.1	see above	5	147	204(72.9)	182
Adjacent 2	$2\ 2^6$, $6\ 6^2$	23	47	70(25.0)	0
Numerical non-disjunction	several	1	5	6(2.1)	0
Total		81	199	280(100.0)	255

Table 1b. Relation between egg-hatch and frequencies of segregation types in testcrosses of T14-heterozygous females (see Table 1a)

Disjunction type	Eggs (n=13175)		Cytological data of Table 1a (n=280)
	Hatched	Non-hatched	
alternate	67%	-	73%
adjacent 1	-	32%	
adjacent 2	-		25%
NND monosomics	-		2%
NND trisomics	1% [1]	-	

[1] Translocation-trisomics die in larval stages; the percentage of trisomics is estimated from the cytological data (1/2 x 2%)

also be derived from the combined data of egg-hatch and cytology (Table 1b). All unbalanced karyotypes, produced by the T14-heterozygotes except the translocation-trisomics arisen by NND, die in the embryonic stages. The egg-hatch observed in testcrosses of T14+ females was on an average 65.6% (n=13175, unfertilized eggs excluded). Because the embryonic lethality in controls was 2-3% and the frequency of trisomics about 1% (cytological data), the frequency of alternate is estimated to be 67%. The percentage of adjacent 1 is then equal to 73 (alt. + adj.1) - 67 = 6.

Metaphases II were used to establish the disjunction types in T14+ males (Table 1a). A striking difference with females is the absence of adjacent 2 and NND . All 255 MII cells scored in ten males corresponded to either alternate or adjacent 1. Because of difficulties in discriminating some of the chromosomes (see above), only 73 cells could be exactly karyotyped. It was, however, absolutely certain that the remaining cells corresponded to either alternate or adjacent 1 because adjacent 2 and NND could be excluded. On the basis of egg-hatch reduction in testcrosses of T14+ males, which amounted to 30.1% (8135 eggs), the percentages of alternate and adjacent 1 are estimated to be 72 and 28 respectively (egg-hatch reduction of controls was 2-3%). These percentages are not significantly different (contingency $\chi^2_1 = 2.9$; $0.05 < p < 0.10$) from the MII scores (n=73) which were 63% and 37% respectively.

Translocations DT_1, DT_3, DT_8 and DT_{11}

After we had found a sex-associated difference in disjunction for translocation T14, four more translocations were studied. Although these translocations had been induced in T14-homozygotes ($2^6 2^6 6^2 6^2$), they can be considered as single reciprocal translocations as they were studied in the T14-homozygous background (see materials and methods).

In translocation DT_1 (Figs. 1 and 3 a,b) as well as DT_3 (Figs 1 and 3d) the breakpoints are located in the short arm of 4 and in the long arm of 2^6. Both translocations have been studied because DT_3-heterozygous females showed significantly lower pupation percentages in the progeny than the corresponding males, whereas such a difference has not been observed for DT_1. In translocation DT_{11} (Figs. 1 and 3 e,f) a small part of the short arm of 2^6 and a long part of the long arm of 5 have interchanged.

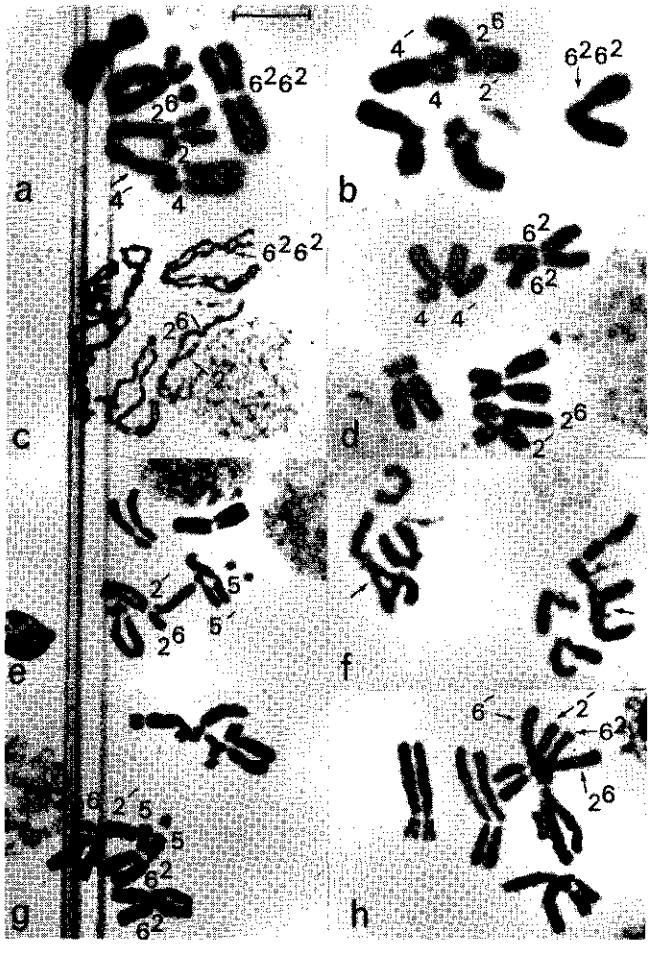

Fig. 3. a-h. Translocation karyotypes of the onion fly; the translocations are induced in T14-homozygous ($2^6 2^- 6^2 6^{2-}$). Note: 2´and 6´ are the retranslocated chromosomes 2^6 and 6^2, 4´and 5´are the other translocated chromosomes. a. DT_1-heterozygote ($2^6 2^- 44^-$),spermatogonial metaphase. b. DT_1-heterozygote, male diakinesis/prometaphase I. c. DT_1, duplication/deficiency embryo ($2^6 2^- 44$). d. DT_3-heterozygote ($2^6 2^- 44^-$), spermatogonial metaphase. e. DT_{11}-heterozygote ($2^6 2^- 55^-$), spermatogonial metaphase. f. DT_{11}-heterozygote, male diakinesis/prometaphase I cells (arrows indicate multivalents). g. DT_{11} duplication/deficiency larva ($2^6 2^- 55$). h. DT_8-heterozygote ($2^6 2^- 6^2 6^-$), spermatogonial metaphase. Bar represents 10 µm.

Table 2. Segregation types and fertility scores in four reciprocal translocations. The fertility and pupation (number of pupae divided by the total number of eggs x 100) were for technical reasons assessed in separate test-crosses.

Translocation	Sex	Number of eggs	Number of MII's	Segregation types (%) Alt.	Adj. 1	Adj. 2	NND	Fertility	Pupation %	Number of eggs
$DT_1(2^6,4)$	♀♀ ♂♂	92	-	[56,5][b]	[42.4][b]	1.1	0	69.4	44.2	611
	♂♂	75	96	57,9	42.1	0	0	71.9	45.8	306
$DT_3(2^6,4)$	♀♀ ♂♂	102	-	[43.1][b]	[56.9][b]	0	0	71.4	22.5	839
	♂♂	120	-	57.5	42.5	0	0	71.2	43.8	468
$DT_8(2^6,6^2)$	♀♀ ♂♂	110	-	[53.2][b]	[43.2][b]	3.6	0	55.8	47.0	298
	♂♂	-	224[a]	49.4	50.6	0	0	48.6	38.4	828
$DT_{11}(2^6,5)$	♀♀ ♂♂	115	-	[48.7][b]	[50.4][b]	0	0.8	68.5	42.6	596
	♂♂	124	180	54.4	45.2	0	0.3	71.5	46.5	1492

[a] 79 MII cells could exactly be classified, the remaining 145 cells were either alternate or adjacent 1 but never adjacent 2 or NDD (see text)

[b] Because of possible interstitial chiasmata these data need not correspond to the disjunction types.

The translocations DT_1, DT_3 and DT_{11} have in common that at least one long interstitial segment is present. In contrast, both breakpoints of translocation DT_8 are proximally located, in the long arm of 2^6 and the short arm of 6^2 (Figs. 1 and 3h). The shape of the DT_8-quadrivalent is very similar to that of T14; the long arm of 2 and the short arm of 6 have changed position, but the lengths of these arms do not differ much. The main difference

between these two translocations is, that the length of the interstitial segments in DT_8 is slightly greater than in T14.

The frequencies of the disjunction types of the four translocations, derived from testcrosses and MII cells, are given in Table 2. It has to be noted that probably often crossing-over has occurred in the long interstitial segments of translocations DT_1, DT_3 and DT_{11}. Therefore, the data from testcrosses of females heterozygous for either of these translocations are not suited to estimate the frequencies of alternate and adjacent 1. These restrictions do not apply to males, as these are achiasmate. Because of rather short interstitial segments it is unknown if and to what extent interstitial chiasmata were present in DT_8-heterozygous females.

In MII cells of DT_8-heterozygous males we have had some difficulties to discriminate between the chromosomes 6^2 and $6'$(the retranslocated 6^2 chromosome) and between 2^6 and $2'$. In total 224 MII cells were scored, but the ratio of alternate to adjacent 1 could only be based on 79 cells. The remaining 145 cells corresponded to either alternate or adjacent 1; adjacent 2 and NND could be excluded.

All four translocations showed predominantly alternate and adjacent 1 segregation. Adjacent 2 was only found in low numbers in females heterozygous for DT_1 (1.1%) and DT_8 (3.6%) but not in males. NND was only observed in DT_{11}-heterozygous females and males in frequencies of 0.8% and 0.3% respectively.

The fertility and the pupation percentages of the translocation heterozygotes, assessed in testcrosses, are given in the right columns of Table 2. The fertility of the translocations DT_1, DT_3 and DT_{11} was significantly higher than the percentages of alternate established in males, indicating that not all duplication/deficiency karyotypes died in the embryonic stage. This was confirmed by the cytological analyses of young larvae where $2^6 2^6 44'$ and $2^6 2'55$ (Fig. 3g) karyotypes respectively appeared to be viable. These karyotypes were not found in older larval stages. In contrast, all duplication/deficiency karyotypes produced by the DT_8-heterozygotes died in the embryonic stage. A conspicuous sex-associated difference in pupation percentage was found for translocation DT_3. The pupation percentages in crosses with heterozygous females were about twice as low as those with males. Afterwards it appeared that the presence of a pericentric inversion in chromosome 5 in addition to the translocation was the reason. This inversion caused no additional mortality in male crosses because of the absence of crossing-over.

DISCUSSION

The most relevant observation of the present study is the pronounced sex-associated difference in segregation behaviour of translocation T14. In males the ratio of alt. : adj.1 : adj.2 was 7:3:0, while in females this ratio was about 8:1:3.

Since in translocation DT_8 the "same" chromosomes (2^6 and 6^2) have rearranged as in T14 and the arm-length ratios of the quadrivalents are almost equal, we suspected also similarities in segregation behaviour. However, in females as well as males great differences in alternate and adjacent 1 frequencies between these two translocations were observed. Also the adjacent 2 frequencies were significantly different, although it is remarkable that both in DT_8 and T14 this segregation type only occurred in females. We suggest that the somewhat greater length of the interstitial segments in translocation DT_8 is the primary reason of the different segregation behaviour. Differences in genetic background affecting the segregation, are supposed to be absent because the heterogeneity between individuals descending from various crosses was low. Information about the frequency of interstitial chiasmata in DT_8-heterozygous females is lacking.

Concerning the translocations DT_1, DT_3 and DT_{11} it is almost certain that frequently crossing-over in interstitial segments has occurred. Therefore, in females the ratios of alternate to adjacent 1 could not be established. In males higher percentages of alternate than of adjacent 1 were found, although only for DT_1 this ratio was (just) significantly different from 1:1 (χ_1^2 = 4.3; $0.025 \leq p \leq 0.05$). With a few exceptions adjacent 2 and NND did not occur.

An important condition for coorientation of centromeres is that the force exerted on a centromere by a pulling spindle-fibre is "stabilized" by a counter-force in opposite direction (Bauer et al., 1961; Henderson and Koch, 1970; Nicklas, 1968, 1971; Sybenga, 1975). Such a stabilization of opposite forces is possible because homologous or partly homologous chromosomes are normally attached to each other, either by meiotic pairing as in males of some dipteran species or by chiasmata. The distance which two centromeres have to cover until a stabilization of the opposite forces occurs, is here indicated as "Coorientation Determining Distance" (CDD). In translocation quadrivalents a distinction must be made between homologous and non-homologous centromeres. The CDD between two homologous centromeres is determined by the distance between the centromeres and the nearest attachment point in an adjacent segment (the non-translocated arm, or the interstitial segment). On the contrary, the CDD between two non-homologous centromeres equals at least the length of the two interstitial segments and the segment of the

interchanged arm from the translocation breakpoint up to the nearest attachment point. Consequently, in general, the CDD's between homologous centromeres will be shorter than those between the non-homologous ones.

It is suggested that differences in segregation behaviour between certain translocations and also between sexes (T14) is mainly caused by differences in CDD's. In prometaphase I of males the chromosomes of normal autosomal bivalents, including the segments in the vicinity of the centromeres, are normally closely associated (Figs. 2 and 3) which will result in very short CDD's between these centromeres. In quadrivalents, because of asynapsis around the pairing-cross (Fig. 2, b-c), it is more difficult to establish how close homologous centromeres are associated. It seems likely, however, that in males the pairing in the non-translocated arms is not affected and therefore also in quadrivalents very short CDD's between homologous centromeres are expected. This is probably the reason that in males homologous centromeres coorientate (segregate) in preference to non-homologous ones, resulting in an absence of adjacent 2. With preferential coorientation of homologous centromeres and in absence of an interaction between the two pairs of homologous centromeres, one would expect a ratio of alt.: adj.1 = 1:1. In T14+ males, however, more than twice as many alternate as adjacent 1 segregations have been observed. When we assume that the orientation of the different centromeres in a quadrivalent tends to occur sequentially, this can be explained as follows. In the first orientation step (almost) always a coorientation of two homologous centromeres takes place because they are close together in T14+ males. Because of this coorientation these centromeres will be situated at a certain distance from each other. The longer this distance the greater the chance that in the next orientation step a coorientation between one of these and one of the other two centromeres occurs, especially when it is not far removed from the first two. This will favour an alternate orientation, because in a three-chromosome orientation only alternate seems to be stable.

Another possibility is that the high frequency of alternate in T14+ males is caused by a relative higher stability (less reorientations) because in an alternate orientation each centromere has two neighbouring centromeres in a trans position (Sybenga, 1975). That an excess of alternate has been observed for T14 but not for DT_8, might be due to the shorter interstitial segments of T14. Because of these shorter interstitial segments already at early stages of the orientation process an interaction between non-homologous centromeres can take place.

In females chiasmata act very probably as the sole reference points for coorientation. Consequently in females with variable positions of chiasmata and short interstitial segments not such pronounced differences in CDD's between homologous and non-homologous centromeres are expected as in males. This is suggested to be the reason that in contrast to males in T14+ females often coorientation between non-homologous centromeres occurs, resulting in the high frequency of 25% adjacent 2. Obviously, for translocations with long interstitial segments (DT_1, DT_3 and DT_{11}) also in females a preferential coorientation of homologous centromeres is expected. Firstly, because longer interstitial segments will automatically lead to on average longer CDD's between non-homologous centromeres and, secondly, because of the presence of interstitial chiasmata. With an interstitial chiasma the homologous centromeres have on both sides a chiasma which may result in shorter CDD's between these centromeres. The data obtained for translocation DT_8 support the present hypothesis. The interstitial segments of translocation DT_8 were somewhat longer than those of T14 but short in comparison to the other three translocations. The same order has been observed for the frequency of adjacent 2, the highest value for T14 (25%), the lowest values for DT_1 (1.1%), DT_3 (0%) and DT_{11} (0%) and an intermediate value for DT_8 (3.6%). The significance of the length of the interstitial segments for segregation has been noticed earlier by Burnham (1950, 1956). For maize translocations he found the same tendency as we have observed for females. According to Wise and Rickards (1977) the presence of interstitial chiasmata would be the main factor which prevents an adjacent 2 segregation. De Boer (1980) concludes just the opposite for T70H heterozygotes in *Mus musculus:* adjacent 2 would be favoured by the presence of an interstitial proximal chiasma. However, the reverse reasoning that the terminilization of an interstitial chiasma is prevented (retarded) by an adjacent 2 disjunction and that the relatively frequent occurrence of adjacent 2 in T70H heterozygotes has other causes, cannot be excluded. Besides, it should be noted that great differences between the T70H translocation and the translocations studied in *H.antiqua* exist, which may have an impact on the way of segregation. Firstly, *M.musculus* has acrocentric chromosomes (interstitial chiasmata are, therefore, a prerequisite for quadrivalent formation) and, secondly, in T70H heterozygotes chains (CIV and CIII + I) were predominantly observed.

Significant differences in segregation behaviour between males and females in *H.antiqua* have been observed for an X-linked translocation as well (van Heemert, 1974, a,b). This was only apparent for the frequency of numerical

non-disjunction (NND), which amounted to 2% in males and 18% in females. Low average chiasma numbers in certain arms of the multivalent has been given as the explanation for the high percentage of NND in females. It has been noted earlier (Vosselman, 1978; Vosselman and van Heemert, 1980) that the sex chromosomes in *H.antiqua* (at least in males) disjoin relatively earlier than the autosomes. This might be the reason that in *H.antiqua* the percentages of NND in sex-linked translocations are on the average higher than in autosomal translocations. In another dipteran species with achiasmate males, *Cohliomyia hominivorax*, translocation-heterozygous males were found which had a higher fertility than the corresponding females (La Chance et al., 1964). The same mechanism as presented here for translocation T14, might have been the basis of this difference.

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FITNESS OF A TRANSLOCATION HOMOZYGOTE IN CAGE EXPERIMENTS WITH THE ONION FLY, *HYLEMYA ANTIQUA* (MEIGEN)

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SUMMARY

In *Hylemya antiqua*, a stock homozygous for an autosomal reciprocal translocation was isolated using egg-hatch reduction and karyotype analysis. Sibling translocation homozygous (TT) and heterozygous (T+) females were compared in respect to egg production and longevity. In one full-sib (5 TT and 8 T+ females) significantly higher values for both parameters for T+ than for TT females were scored, in four others (a total of 35 TT and 28 T+ females) no significant differences were found. Cage experiments were started with populations composed of equal numbers of wild type flies (++) and translocation homozygotes. The frequencies of the different karyotypes in three successive, non-overlapping generations, did nog suggest substantial differences in fitness between ++ and TT flies. Possible causes of a surplus of T+ individuals found in these experiments are discussed together with the usefulness of the translocation for genetic control of *H. antiqua*

INTRODUCTION

Various types of genetic control for insect pests have been proposed (reviews by Smith and von Borstel, 1972; Davidson, 1974; Whitten and Foster, 1975). One of these is to utilize chromosomal translocations (Robinson, 1976). To develop a genetic control method for the onion fly, Hylemya antiqua, in the Netherlands two different approaches were chosen: the sterile male technique (Loosjes, 1976) and methods involving chromosomal rearrangements (van Heemert, 1975, 1977; van Heemert and Wijnands-Stäb, 1975; Robinson and van Heemert, 1975; Wijnands-Stäb and van Heemert, 1974).

The genetic load induced in a population by introducing translocations depends on the production of unbalanced (deficiences and duplications) gametes by the translocation heterozygotes (T+). Translocation homozygotes (TT) are expected to be fully fertile when crossed with each other or when crossed with individuals of the normal karyotype (++). A consequence of the partial sterility of the translocation heterozygotes is that,in general, no stable equilibrium for the different karyotypes in a population can be achieved. However in certain cases, when the partial sterility of the heterozygotes is at least 80% and there is sufficient complementation of unbalanced gametes in T+ x T+ crosses, such a stable equilibrium is possible (Stam, 1979). The level of partial sterility necessary to reduce a population below the economic injury level has to be established experimentally because density-dependent and other factors may have an adverse effect.

Another application of translocations

in insect control is the manipulation of gene frequencies in a population by using the translocation as a transport mechanism (cf. Curtis, 1968; Whitten, 1971). In laboratory-experiments Feldmann (1979) has tested three different strains of *Tetranychus urticae* homozygous for a structural chromosome mutation (T), for their ability to replace a standard strain. A population replacement could be realized for two strains when the initial frequency of the "T-karyotype" was at least 0.65.

For both methods an essential requirement is that the translocation-homozygotes have a good fitness. However, in many insects it has been demonstrated that radiation induced translocations are often either lethal or show severe fitness reductions when made homozygous (review by Robinson, 1976; Reid and Wehrhahn, 1976; Baker et al., 1977; van Heemert, 1977; McDonald et al., 1978). In the onion fly three out of eight different translocations appeared to be viable as homozygotes in the adult stage. Recently a few more translocations with viable homozygotes have been isolated, but so far it has not been possible to establish stable pure breeding homozygous stocks,probably due to reduced fitness of the homozygotes(van Heemert and Robinson, personal communication), with the exception of the one presented here.

There are many reports about cage experiments with translocation heterozygotes, but only a few dealing with homozygotes (Robinson and Curtis, 1973; Reid and Wehrhahn, 1976; Feldmann, 1979).

The object of this study was to assess the fitness of flies homozygous for an autosomal reciprocal translocation under competitive conditions. In separate experiments longevity and egg production of homozygous females were compared with heterozygous females.

MATERIALS AND METHODS

Isolation of translocation homozygotes

Seven days old wild type (++) males were irradiated by a dose of 200 rad X-rays and mated with ++ females. Translocation T14 was isolated from a F_1 female which showed a reduced egg-hatch. To remove radiation damage and to obtain genetically diverse material, translocation heterozygous (T+) females were backcrossed with ++ males for at least five generations, in mass cages with excess ++ males. Males were not used as backcross parents (T+), as they are achiasmate (no recombination for loci on the translocated chromosomes). After mating, females were separated and each progeny was reared separately. The adults from these progenies were mated in separate cages to finally obtain several "lines" with a low kinship between lines. Egg-hatch reduction was always used as selection criterium to identify the T+ x ++ crosses. Classification of eggs is simple, the unbalanced karyotypes from T14 die as embryos and can be recognized by a brown colour of the eggs; unfertilized eggs remain white.

After intercrossing different "lines" at the 5^{th} backcross generation (G_6), T+ x T+ progenies were selected on the basis of egg-hatch reduction and karyotype analysis. The crosses made in G_6 and the following generations correspond in main lines with the scheme given in Fig. 1.

In G_7, males (from T+ x T+ crosses) were testcrossed with ++ females and the TT males could be identified by karyotyping one larva from those crosses with a percentage of brown eggs of 0-4% (++ x ++ and ++ x TT). Six TT males were selected in this way and they were subsequently mass-mated with females (++, T+ and TT) in two cages (3 males/cage). From each mass cage at least one TT x TT progeny was selected, together with some TT x T+ progenies (not shown in Fig. 1). In G_8 some TT x T+ progenies were obtained by direct crossing of T+ x T+ progenies, i.e. without first testcrossing the males. Adult flies of G_8, from either TT x TT or TT x T+ crosses, were outcrossed with T+ to reduce inbreeding effects in the next generations. These T+ parents descended from different backcross "lines" (T+ x ++). In G_9 TT x T+ progenies were selected (on the basis of egg-hatch and partially by karyotype analysis) and finally by crossing these, in G_{10}, several genetically different TT x TT progenies were isolated; this was confirmed by cytology.

In order to rear on a large scale flies (as larvae) for the cage experiments, the adults of G_{10} were mated in groups according to the degree of kinship. For each combination the inbreeding coefficient I (I meaning the

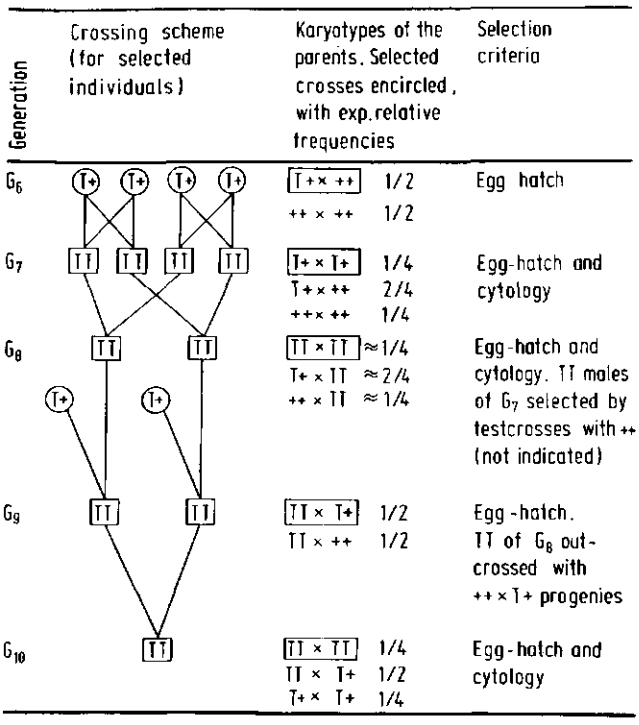

Fig. 1. Basic scheme used for the isolation of homozygotes (TT) for translocation T14 of *H. antiqua*, after five generations of backcrosssing of translocation heterozygotes (T+) with wild types (++).

chance that the two alleles for each locus of an individual are both a copy of the same ancestral allele) was calculated. In this way in G_{11} four groups of TT individuals (a, b, c and d) with an average inbreeding coefficient of 0.04 (range 0.03-0.05) were obtained; these were used for the cage experiments.

In G_{11} all larvae were reared on an artificial medium (Loosjes, 1976), because this made it easier to rear high numbers under standardized conditions. In preceeding generations the larvae were reared on onion (Vosselman 1978).

Female longevity and egg production

To compare TT and T+ females in respect of longevity and total egg production, progenies of five different TT x T+ crosses were used. These females were reared as larvae on onion, during the first 3-4 days in the dark at a temperature of 25 °C, and subsequently at 20 °C and 16 h daylight. The adult flies were kept at 23 °C, approximately 70% r.h. and 16 h daylight. Eggs were collected during the first weeks once every three days and later once every four days. Females were only stimulated to lay eggs for 48 hours previous to the time of collection (Vosselman, 1978), by providing them with a slice of raw onion.

Experimental design of cage experiments

The initial population comprised 3,000 flies, divided over six cages. In each cage 125 ++ ♀♀, 125 TT ♀♀, 125 ++ ♂♂ and 125 TT ♂♂. The origin of the ++ population used in this experiment was different from the ++ flies used for the backcrosses of T+, in so far that the pupae were collected from different places in the southwest of the Netherlands. The four groups of TT individuals of G_{11} (see above) were sexed and divided between the cages in such a way that in half of the cages only a x b and in the other half only c x d crosses were possible. The flies were of the same age and placed together one or two days after eclosion of the pupae. For three weeks, when the flies were 14-35 days old, eggs were collected a total of four times. The number of eggs used for rearing the larvae amounted to 40,000-50,000. The number of pupae obtained was 25,000-35,000. As much as possible equal numbers of eggs per cage were taken. The larvae of the cages with a x b crosses were reared separately from those of the cages with c x d crosses.

A number of 3500-4000 adult flies of G_1, divided over 8 cages were used for rearing the second generation. The inbreeding coefficients of the TT individuals were again on an average 0.04 (0.03-0.05). The flies were not sexed (pupae were placed in the cages) and each cage consisted half of flies of the a x b group and half of the c x d group. Egg collection and larval rearing was equal to the previous generation. The third generation of flies was obtained in the same way as the second one, except for the division into the different groups.

During all these experiments adult flies were reared in cages of size 25 x 25 x 25 cm, at 20 °C, 70% r.h. and 16 h daylight. For rearing conditions of the larvae and the composition of the larval rearing medium see Loosjes (1976).

As it is more difficult to karyotype females, almost exclusively males were scored, either in the pupal stage or one day after eclosion of the pupae. The cytological techniques have been reported earlier (Vosselman,1978).

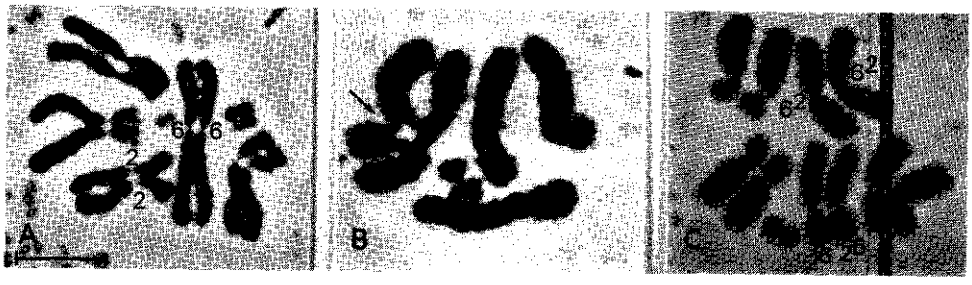

Fig. 2. Karyotypes of *H. antiqua*. A - spermatogonial metaphase of ++ male (2266); B - prometaphase I of T+ male ($2^6 2\ 6^2 6$); C - spermatogonial metaphase of TT male ($2^6 2^6 6^2 6^2$).

RESULTS

Cytology and fertility of the translocation

The breakpoints of translocation T14 are located in the long arms of the autosomes 2 and 6, near the centromeres (Fig. 2). The translocated chromosome 2^6 can be recognized because it is slightly longer than chromosomes 3 and 4, while chromosome 2 is the smallest autosome. Chromosome 6^2 is shorter than 6 and almost metacentric. The difference in length of the exchanged chromosome segments is sufficient to distinguish TT from ++ individuals, at least in testis preparations. In preparations of embryos, larval brains or ovaria, with in general a lower number of scorable cells and less contracted chromosomes, the disparity was not always clear. Therefore, predominately males were classified. The T+ individuals are recognizable in all stages due to the presence of a quadrivalent configuration either in somatic or meiotic pairing (Fig. 2).

During backcrossing of T+ females a considerable variation between crosses in the number of dead embryos (brown eggs) was observed. It could not be determined if this was caused by genetic differences. The average percentage of brown eggs in T+ (♀) x ++ (♂) crosses amounted to 34.4 (13175 eggs). In ++ (♀) x T+ (♂) an average percentage of 30.1 (8135 eggs) was found. This difference could be expected, because the frequencies of the disjunction types of the translocation multivalent were not equal for both sexes (Vosselman, in prep.).

Female longevity and egg production

Preceding the cage experiments, TT and T+ females were compared with respect to longevity and total egg production (Table 1). Progenies of five different TT x T+ crosses were used. The results of four of these crosses (35 TT and 28 T+ females) have been combined since the average values for longevity and egg production of the TT females were not different from T+. In one progeny (cross 5) the T+ females had significantly higher values for longevity ($F^1_{11} = 6.9$; $0.01 < p < 0.05$) and egg production ($F^1_{11} = 14.8$; $p < 0.01$).
The variation in number of eggs and longevity of the TT females of cross 5 was remarkably low.

Table 1. Egg production and longevity of sibling females homozygous (TT) and heterozygous (T+) for translocation T14 in *H. antiqua*. These females descended from five different TT x T+ crosses, the results of one of which, with significant differences between TT and T+ have been presented separately.

Crosses	Female karyotype	Number of females	Number of eggs /female		Longevity (days)	
			mean ± S.E.	range	mean ± S.E.	range
1,2,3,4	TT	35	456.4 ± 144.7	213-859	52.4 ± 14.4	27-84
1,2,3,4	T+	28	483.6 ± 169.1	168-893	56.3 ± 19.7	23-96
5	TT	5	469.8 ± 43.3[a]	441-545	43.8 ± 6.7[b]	32-48
5	T+	8	817.5 ± 195.9[a]	438-1067	67.6 ± 19.3[b]	30-82

a $F_{11}^{1} = 14.8$, $p < 0.01$ (analysis of variance)

b $F_{11}^{1} = 6.9$, $0.01 < p < 0.05$

Cage experiments

The frequencies of karyotypes observed in three succesive generations are given in Table 2 starting from a parental population consisting of half TT and half ++ individuals. Assuming that TT and ++ individuals had an equal fitness, one would expect in generation G_1 a ratio of 1:2:1 for ++, T+ and TT respectively. In the following generations complementation of unbalanced gametes in T+ x T+ crosses could occur, but its frequency will have been low because of differences in disjunction of the translocation multivalent between the sexes. In males only adjacent 1 and alternate orientations occur, while in female adjacent 2 and alternate orientations predominate (Vosselman, in prep.). The estimated increase in number of T+ individuals in generations G_2 and G_3 due to complementation, therefore, amounts to only 0.5-1.0%.

For reasons mentioned above males were predominately used to establish the karyotype frequencies but in generation G_3 41 females were also karyotyped. As there were no significant differences between females and males the results for both sexes have not been presented separately in Table 2.

The percentage of brown eggs (dead embryos) in generation G_1 was 1.2 (805 eggs), in G_2 32.1 (2179 eggs) and in G_3 36.2 (2317 eggs).

To establish the karyotype frequencies, pupae and newly emerged males were used. For the emerged males it could be determined if differences in time of eclosion between karyotypes were present. The period between the first and last emerging males in all generations varied from 2 to 4 days, but the majority of the males emerged in two days in about equal numbers per day. The males were split in two groups (Table 3), group 1 representing the first emerging "half" and group 2 the last emerging "half". Because the eclosion of pupae occurs in a short period during the night or in the morning, it was not possible to divide the males in two groups with exactly the same numbers. A general tendency was observed to be a surplus of T+ and a shortage of TT in the first emerging group of males. Apparently the pre-adult development time of the TT males was on the average somewhat longer than of T+. The data for the ++ males are more difficult to interpret, but it is suggested that the ++ males had a pre-adult development time in between T+ and TT, at least in generations G_1 and G_2.

Table 2. Karyotype frequencies in adults of *H. antiqua* observed in three successive generations starting from an initial mixed population of wild type flies (++) and translocation homozygotes (TT) in a ratio of 1 : 1.

Karyotypes	Numbers (%) of karyotypes in generation:		
	G_1	G_2	G_3
++	36 (23.1)	34 (19.8)	32 (23.0)
T+	81 (51.9)	96 (55.8)	83 (59.7)
TT	39 (25.0)	42 (24.4)	24 (17.3)
Total	156	172	139
Not scorable	4	5	4

Table 3. Time of eclosion of male pupae of *H. antiqua* of Table 2. The males have been split in two groups in sequence of emergence. Group 1 represents the first emerging "half" and group 2 the last emerging "half" (see text).

Karyotypes	Percentages of karyotypes in each group/generation								
	G_1			G_2			G_3		
	Group 1	Group 2	Ratio[a]	Group 1	Group 2	Ratio[a]	Group 1	Group 2	Ratio[a]
++	22.9	28.6	0.8	17.5	20.3	0.9	24.5	20.0	1.2
T+	65.7	38.8	1.7	71.4	46.4	1.5	64.2	53.3	1.2
TT	11.4	32.7	0.3	11.1	33.3	0.3	11.3	26.7	0.4
Number	35	49		63	69		53	45	

a $\frac{\text{group 1}}{\text{group 2}}$

DISCUSSION

Female longevity and egg production

The average values for longevity and egg production for ++ females from both a recently colonized and from a laboratory strain of *H. antiqua* obtained by Robinson and Zurlini (1979) with about the same rearing conditions, were somewhat lower than the present values, indicating that the chromosomal rearrangement T14 does not negatively affect these two characters.

However, in one full-sib a significantly higher egg production and adult longevity for T+ compared to TT females were observed (Table 1). This is presumably due to genotypic differences and not due to the chromosomal rearrangement per se, as in four other full-sibs no significant differences were found. Such genotypic differences between the TT and T+ females could be expected since the male parent of these females was T+ and males are achiasmate. Because of the absence of crossing-over genotypic differences were possible for all loci on the chromosomes involved in the translocation which were heterozygous in the male parent. As egg production is related to female longevity it is not necessary to assume that the differences measured for these characters are due to genotypic variation for different loci.

Another conclusion from the present data might be that for determining the effect of chromosomal rearrangements on fitness, it is important to use genetically diverse material to avoid a disturbing effect of linked factors.

Cage experiments

The data obtained from the cage experiments indicated no substantial differences in fitness between the TT and the ++ flies. In generation G_3 somewhat more ++ than TT individuals were found, but in G_1 and G_2 the opposite was observed.

In all three generations the observed numbers of T+ individuals were slightly higher than expected, but only in generation G_3 was the ratio of T+: ++ and TT significantly different from 1 : 1 ($\chi^2_1 = 5.2$, $0.02 < p < 0.05$). In fact as a consequence of complementation of unbalanced gametes in T + x T+ crosses in G_2 and G_3, the expected percentages of T+ should have been 0.5-1.0% higher, but even taking this into account an excess of T+ individuals is apparent. The T+ individuals probably had a selective advantage in some way, perhaps manifested by the on average earlier eclosion of the male T+ pupae (Table 3). In the plants *Campanula persicifolia* (Darlington and La Cour, 1950) and *Clarkia speciosa* (Bloom, 1977) translocation heterozygotes can be at a

selective advantage under conditions of inbreeding. Also, John and Lewis (1958, 1959) have attributed the presence of unexpected high frequencies of translocation heterozygotes in some populations of the cockroach *Periplaneta americana* and *Blaberus discoidalis* to inbreeding.
Preservation of genic heterozygosity has been generally suggested to be the cause of excess translocation heterozygotes. As the inbreeding coefficients in the present experiments were low, it is questionable if inbreeding was the reason of the excess of T+.

Because all T+ individuals in generation G_1 were hybrids between ++ and TT, a higher level of genic heterozygosity of the T+ (resulting in a selective advantage) might be a consequence of differences in gene frequencies between the parental ++ and TT population. In that case, however, recombination in T+ individuals (males achiasmate) would lead to smaller differences in genic heterozygosity and therefore also a smaller excess of T+ was expected in generations G_2 and G_3. The data did not suggest a decrease but an increase in the relative frequency of T+ from G_1 to G_3. Apparently other unknown factors have attributed to the excess of T+ individuals.

Hitherto only a few reports about cage experiments incorporating translocation homozygotes have been published, although such experiments are indespensable to ascertain the overall fitness of the homozygotes.
Robinson and Curtis (1973) established for a translocation in *Drosophila melanogaster* that the homozygotes had practically normal fertility and viability in non-competitive conditions. On the contrary, in cage experiments the translocation homozygotes were rapidly eliminated even when the initial mixed population consisted of 9 times as many TT as ++. Four different homozygous-viable translocations in *Drosophila melanogaster* were tested by Reid and Wehrhahn (1976). In small scale experiments they found that starting from initial ratios of 9:1 for TT and ++, for two translocation there was no increase in the relative frequency of non-translocated chromosomes after one month. However, a period of one month is too short to draw conclusions about the competitiveness of the TT individuals. In addition, it should be noted that only 21 of 57 translocations were viable as homozygotes and the four used in the cage experiments were selected among the best half with respect to "homozygote viability". Attempts to replace indigenous populations of *Aedes aegypti* in Kenya by a translocation homozygous strain were not successful (Lorimer et al., 1976). The homozygotes appeared to be deficient in several fitness factors.

Isolation and viability of homozygotes

When markers are available, "pseudo-linkage" methods can be used to isolate translocation homozygotes (Robinson, 1976; McDonald et al., 1978). With such methods an efficient selection of desired karyotypes is possible. In *H. antiqua* suitable markers are lacking and therefore a method relying on egg-hatch reduction combined with karyotype-analysis has to be used. Recently Reid and McEwen (1977) proposed a method for *H. antiqua*, based solely on reduction in egg-hatchability. Such procedure is, however, much more cumbersome because to establish the viability of the homozygotes and to select the desired crossing combinations, several testcrosses are necessary. Further, egg-hatch reduction is not a very reliable parameter, because a considerable variation can occur due to environmental or genetic factors (Dennhöfer, 1975; Hossain et al., 1974; Hossain and Curtis, 1975) Reid and McEwen suggested also to use the level of egg-hatch reduction of T+ x T+ crosses as an indication for the viability of the translocation homozygotes. However, when no information about the degree of complementation of unbalanced gametes is available, this can lead to wrong conclusions.

It is known from experiments of Robinson (1977) and of own unpublished data, that inbreeding in *H. antiqua* can result in increased mortality, particular in the larval stage. Therefore, in order to obtain sufficient genetic variation, particularly for the chromosomes under selection (the translocated chromosomes), and to remove radiation damage the translocation heterozygotes were backcrossed at least five times with wild type flies (++). Because males are achiasmate females were always used as the backcross parent. This is in contrast to other results where in order to maintain a linkage between translocation breakpoints and markers many workers have used achiasmatic males as backcross parent (e.g. Wagoner et al., 1969; McDonald and Overland, 1973a, b; Foster and Whitten, 1974; Reid and Wehrhahn, 1976). It is obvious, that in these cases the translocation-homozygotes will have an increased chance of being inviable or less fit, due to the presence of higher frequencies of homozygous loci, at least for those loci located on the translocated chromosomes.

Ytterborn (1970) has shown in *Drosophila melanogaster* that the percentage of translocations viable as homozygotes was inversely related to the radiation dose applied for the induction of the translocations. With a dose of 500 rad the percentage of translocations viable as homozygotes was about four times as high as with a dose of 3500 rad. In the present case only 200 rad of X-rays were used.

Significance for application

In a preliminary field cage experiment it has been established that not only the T14 translocation homozygotes but also a ++ stock maintained for six generations in the laboratory, show higher diapause sensitivity in comparison with natural populations of *H. antiqua* (Robinson et al.,1979). This illustrates the caution that must be applied when extrapolating from laboratory experiments to the field situation.

The significance of translocation T14 for genetic control purposes seems to be limited, because the genetic load which can be induced amounts only to 30-35%. For other reasons too, this translocation does not meet the requirements for a stable equilibrium (Stam, 1979). Further, considering the high variation in egg-hatch between the translocation heterozygotes, it cannot be excluded that under selection the egg-hatch will increase. Such an increase in fertility has been demonstrated e.g. in *Drosophila melanogaster* (Hossain et al., 1974), *Musca domestica* (Hossain and Curtis, 1975) and in some plants (Sybenga, 1975). Dennhöfer (1975) also found evidence for genetically determined differences in segregation for a translocation in *Culex pipiens*.

However, translocation T14 could be very useful for population replacement or when it is used in combination with other chromosomal translocations. In order to obtain translocations with a higher genetic load, double-translocations have been induced by irradiating some T14 homozygotes. In particular, three-chromosome double-translocations have been studied, because these can have some advantages above four-chromosome double-translocations,or three-chromosome double-translocations obtained by combination of single ones (Vosselman, in prep.). To be able to isolate such double-translocation homozygotes with a good fitness, it is obvious that a re-irradiation makes sense only for translocation homozygotes with a fitness comparable with wild type flies.

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Genetic control of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). Sensitivity to diapause interfering with a field-cage experiment using a homozygous chromosomal translocation

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Abstract

A translocation homozygous strain (*TT*) of *Delia antiqua* (Mg) was released into a field cage in Wageningen, The Netherlands, together with a standard laboratory strain (++). During the course of the season, the fecundity and fertility of the adults were measured together with the karyotype frequencies (*TT* ; *T*+; ++) of the F_1 progeny. No selective disadvantage of the translocation karyotypes was observed. However, only four F_1 adults emerged in the field cage; therefore a sample of pupae was removed from the cage, and it was shown that nearly 100% of the surviving pupae had entered diapause. This figure was confirmed from the remainder of the pupae. The fertility of eggs from the emerging adults was reduced to 54%, compared with the standard fertility of nearly 90%, but because of the diapause response of the strains used, the effect of this reduced fertility in the field-cage population could not be followed. The reasons for the change in diapause response of the laboratory strains are discussed and suggestions made as to how this could be prevented. The report highlights the importance of quality in control techniques involving translocations.

Introduction

The reduction in the fertility of populations caused by translocations was suggested by Serebrovski (1940) as a means of controlling insect pests. The idea was further developed independently by Curtis (1968). As in many fields, the theory has far outstripped the practice and to date there have been relatively few field or field-cage tests to assess the effect of translocation releases on the dynamics of pest populations. Field experiments have been reported on mosquitoes (Laven, 1969; Cousserans & Guille, 1974; Seawright et al., 1975; Curtis et al., 1976; Lorimer et al., 1976; Suguna et al., 1977; Terwedow et al., 1977), house-flies (Wagoner et al., 1973; Wagoner et al., 1976) and the sheep blowfly, *Lucilia cuprina* (Wiedemann) (Foster et al., 1978). In all these experiments, with the exception of Lorimer et al. (1976), strains with heterozygous translocations were released. The advantage of such releases is that there is an immediate effect on the current egg fertility in the population, but the disadvantage

is that for every translocated chromosome released, a normal chromosome is also released. If translocation homozygotes are released, then a much higher translocation frequency can be injected into the population together with a much longer term genetic load. Further, if population replacement is to be attempted, then translocation homozygotes have to be released.

In the onion fly, *Delia antiqua* (Meigen), radiation-induced translocations have been produced and isolated (Wijnands-Stäb & van Heemert, 1974; van Heemert & Wijnands-Stäb, 1975; Robinson & van Heemert, 1975; Robinson, 1977) and many have been inbred in order to isolate homozygotes. As in most insect species, the frequency with which translocations can be made homozygous is low (Robinson, 1976). Nevertheless, in two cases, one sex-linked (van Heemert, 1977) and one autosomal (Vosselman, in press), homozygous stocks have been reared.

In 1977, a field-cage system was developed at the Institute for the Use of Atomic Energy in Agriculture, Wageningen in the Netherlands, in which it was possible to maintain a population of *D. antiqua* under an approximation of field conditions and in which several population parameters could be measured. In 1978, it was decided to utilise this system to observe the effect that the release of an autosomal homozygous translocation could have on the build-up of populations of *D. antiqua*. A second aim was to assess the fitness of the homozygotes under field conditions and to compare this with the results obtained in cage studies with laboratory populations (Vosselman, in press). *D. antiqua* is multivoltine, and diapause is facultative. Normally there are two generations a year, and in the first generation approximately 21% of individuals enter diapause compared with nearly 90% in the second generation (Loosjes, 1976).

Materials and methods

Strains

Translocation homozygote T/T. This was induced in sperm by irradiation of males with 200 rad X-rays. The method used to isolate the homozygote will be published elsewhere (Vosselman in press). The translocation is between the long arms of chromosomes 2 and 6. The exchanged pieces are rather similar in length, but in testes preparations it is possible to distinguish the homozygote from the normal karyotype (Plate IV*a*, *b*). The heterozygote is easily identified by the presence of a quadrivalent (Plate IV*c*). The translocation strain was backcrossed for several generations to a recently colonised (4–5 generations) standard strain. Subsequently, it was reared as a homozygote for a further 5–6 generations.

Standard strain $+/+$. This is the normal karyotype (Plate IV*a*) and is not genetically identical with that used for backcrossing to the translocation. The strain had been reared in the laboratory for 6 generations.

Field cages

Adult flies that had emerged in the laboratory (P generation) were introduced into two cages situated within the grounds of the Institute in Wageningen on 16 May 1978. The cages had dimensions of $150\times100\times100$ cm, and into each cage a total of 400 adult virgin flies was released with equal numbers of the T/T and $+/+$ strains and equal numbers of both sexes. The flies were provided with standard *D. antiqua* food (Ticheler, 1971), and oviposition sites were provided in the form of young onion seedlings in pots (4/cage). Twice weekly the pots were brought to the laboratory and the total number of eggs per cage was counted.

The eggs were then returned to a smaller larval rearing cage (50×50 cm), which was placed so that the bottom of the box was 20 cm below ground surface level. The cage was sealed at the bottom to prevent emigration, so that the total number of pupae could be counted. Onion seedlings were placed in the cage as food for the growing larvae. As the larvae grew, extra food was provided in the form of half onions.

Following eclosion of the adults in this small cage, they were counted and returned to the large cages to lay eggs to start the second generations.

Egg samples

Two egg samples from each cage (A–D) were taken during the period of oviposition to assess their fertility and the karyotype. The eggs were placed on moist filter paper on top of a slice of onion and incubated at 29°C for 2 days. Subsequently, they were classified as hatched, white (unfertilised) or brown (late embryonic lethals). The larvae hatching were reared at 23°C, and the karyotype of both males and females of the F_1 adults was assessed.

Results

Oviposition

The first eggs from both cages were collected on 29 May, that is after a pre-oviposition period of 13 days. The cumulative percentage egg production curves for both groups of flies (Fig. 1) follow a parallel pattern with cage II producing the greater

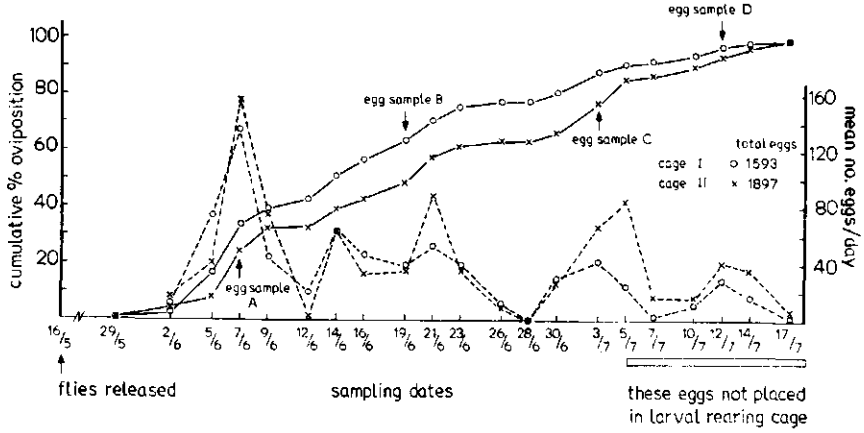

Fig. 1.—Overall fecundity and the oviposition rate in two field cages of *D. antiqua*.

number of eggs. It was observed that the egg production per day was very dependent on weather conditions (Fig. 1). The mean number of eggs produced per female was 7·96 and 9·48 for cages I and II, respectively, a much lower figure than has been recorded previously either in field cages (Perron & Lafrance, 1961) or in the field (Loosjes, 1976). The reasons for this low fecundity are unclear. The eggs produced at the end of the oviposition period were not transferred to the larval rearing cage; a total of 2726 eggs was transferred.

Egg samples

Four samples (2 from each cage) of 50 eggs were taken during the oviposition period (Fig. 1). The data from these are shown in Table I. As all individuals in the cage were homozygotes, either standard or translocation, the egg hatch was close to normal, with only the last egg sample showing an appreciable reduction in fertility. The overall observed distribution of karyotypes in the emerging males and females indicated no deviation from the expected distribution of $1T/T:2T/+:1+/+$ ($\chi_2^2=4{\cdot}66$; $P>0{\cdot}05$).

TABLE I. *Fertility of eggs, larval and pupal survival and adult karyotypes in four egg samples (A–D) taken from two field cages of* D. antiqua *

Sample	Total no. of eggs	No. of full white eggs	No. of full brown eggs	Larvae No.	Larvae (%)	No. (%) of pupae	Adults no. (%)	Karyotype of F_1 adults: Males +/+	Males T/+	Males T/T	Males ?	Females +/+	Females T/+	Females T/T	Females ?
A	50	3	2	45	(90)	39 (89·7)	35 (89·7)	7	9	5	1	1	7	3	2
B	50	8	3	39	(78)	33 (86·6)	22 (66·7)	1	4	4	2	1	4	1	5
C	50	8	1	41	(82)	34 (82·9)	29 (85·3)	1	9	0	3	0	7	0	9
D	50	9	7	34	(68)	25 (73·5)	22 (88·0)	3	8	3	1	0	1	0	6
							Total	12	30	12	7	2	19	4	22

*The cages were started with a 1:1 ratio of translocation homozygotes (*T*/*T*) to standard (+/+).

However, several differences can be seen between the sexes and between the samples. First, the 'uncertain' column for the females contains nearly 50% of the total examined. The equivalent value for males is 11%. This is because the preparation of female chromosomes is more difficult than for males and also because a proportion of these preparations could not be analysed for technical reasons. Further, with this translocation, the difference in the pieces exchanged is not very great. Therefore, although the heterozygote can be readily distinguished, differentiation between the two homozygotes, $+/+$ and T/T, can only be achieved with good preparations. Due to these problems, the karyotype distribution in the females is significantly biased in favour of the heterozygotes ($\chi_2^2=7{\cdot}08$; $P<0{\cdot}05$). The overall sex ratio was not significantly different from 1:1 ($\chi_1^2=1{\cdot}82$; $P>0{\cdot}1$). The overall fitness of the translocation homozygotes in the field could not be assessed as the parents of the immature stages were reared in the laboratory. The individuals used to determine the karyotype frequencies were also reared under laboratory conditions. However, there appears to be no selective disadvantage for the translocation homozygotes under field conditions with regard to mating competitiveness, egg production and adult life-span.

Larval survival and the incidence of pupal diapause

A total of 2726 eggs was transferred to the larval rearing cage and, as the mean hatch in the laboratory of the four egg samples was 83%, then under optimal conditions

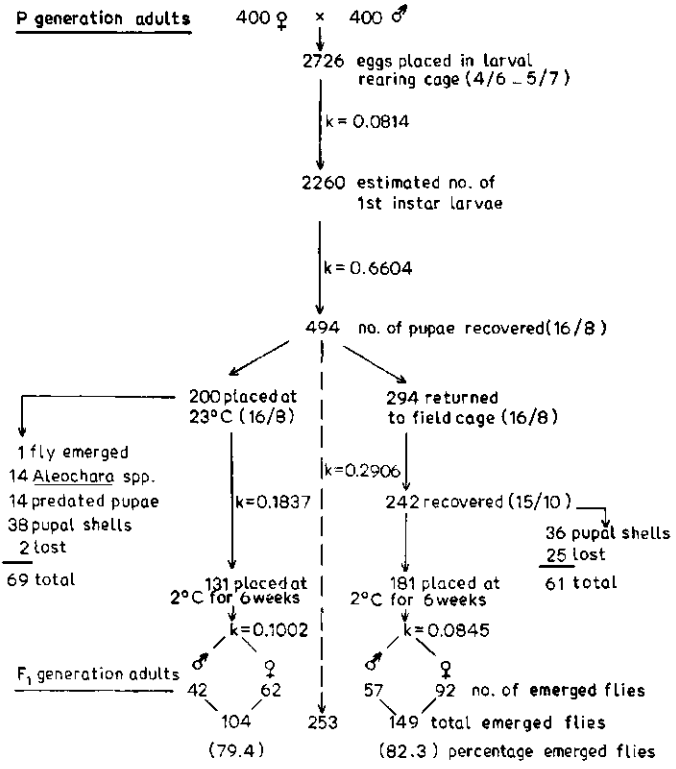

Fig 2.--Schematic representation of larval and pupal survival and pupal diapause in *D. antiqua* in a field cage.

the emergence of 2260 larvae could be expected. During the course of the larval development, despite the extra half onions placed in the cage to supplement the onion seedlings, the larval feeding system was less than optimal. This is well illustrated if the k values (Varley & Gradwell, 1960) for the developmental stages are calculated (Fig. 2); it is apparent that larval mortality contributes most of the total mortality. The emergence of the second generation adults was expected in late July–early August, but by then only 2 males and 2 females had emerged. It was therefore decided to make a count of the pupae in the cage. All the soil was removed, and a total of 494 pupae was recovered, giving an approximate larval survival of 22%. Two hundred of these pupae were placed in the laboratory at 23°C to check emergence, and the remaining 294 were returned to the field cage. Within two weeks, one *D. antiqua* emerged from the 200 pupae and beetles identified as *Aleochara* spp. emerged from 14 pupae. A further 14 pupae were parasitised and there were 38 empty pupal shells; 2 pupae were lost.

The remaining 131 pupae were maintained at 2°C for 6 weeks in order to break diapause and then at 23°C. A total of 42 males and 62 females emerged, indicating that in the first generation at least 79% of pupae had entered diapause. Following this unexpected finding, the pupae in the field cage were again counted and 242 were found. Thirty-six of these were empty pupal shells and of the remaining pupae, 181 were kept at 2°C for 6 weeks and then at 23°C. A total of 57 males and 92 females emerged, giving an incidence of pupal diapause of at least 82·3% (Fig. 2). Calculated k values for eggs, larvae and pupae were 0·0814, 0·6604 and 0·2906, respectively. Pupal mortality can be further partitioned to illustrate the importance of predators (k=0·1837).

Egg fertility in the F_1 generation

Following the emergence of the second group of flies in the laboratory, random mating was allowed and the fertility of this population was assessed under laboratory conditions. The fertility of eggs was reduced to 54% (Table II) compared with the

TABLE II. *Fertility of eggs in the F_1 generation of a* D. antiqua *population following random mating*

Date	Total no. of eggs	No. of full white eggs	No. of full brown eggs	No. of larvae
18.xii	100	4	42	54
22.xii	100	3	35	62
27.xii	100	14	39	47
Totals	300	21	116	163
Percentages		7·0	38·7	54·3

fertility in the parental generation of 80% (Table I). It was extremely unfortunate that the diapause sensitivity prevented observations of the effect that such a reduction in fertility could have on population growth in the field cage.

Discussion

As a consequence of the sensitivity to diapause induction of the genomes of both strains used in the experiment it was not possible to assess the effect of inherited semi-sterility on a population of *D. antiqua* maintained under field conditions. Nevertheless, it was shown that (1) random mating occurred between the standard and the translocation homozygous individuals in the field cage, (2) survival under laboratory conditions from egg to adult of the translocation homozygote, the heterozygote and the standard did not differ and (3) following random mating of the F_1 adults in the

laboratory the population fertility was reduced by about a half compared with the P generation. These results are in good correspondence with the data from cage studies on a laboratory population (Vosselman, in press).

The interference by diapause sensitivity was unexpected and certainly represents a hurdle which has to be overcome if such translocation strains are to be used for population control of *D. antiqua*. This species is multivoltine. Diapause is facultative and its induction is strongly age-dependent, with short daylength inducing diapause in third-instar larvae (Ramakers, 1973) and low temperature inducing diapause in the first few days of pupal development (Kelderman, 1972). Data from eight field experiments indicate that between 0 and 29% of individuals in the first generation enter diapause (Loosjes, 1976), whereas in the present experiment nearly 100% of individuals entered diapause, a clearly significant increase.

It is suggested that continuous rearing in the laboratory under non-diapause conditions has resulted in this change in diapause sensitivity. In the field, there will be selection against diapause in the first generation as such diapause individuals will not take part in the second generation. Nevertheless, the fact that in field populations a proportion of first-generation individuals continue to enter diapause indicates that there must be some form of balancing selection operating. When this selection pressure is completely removed during laboratory rearing, then sensitivity to diapause will increase. The precise manner in which the relaxation of selection operates is not clear; perhaps diapause sensitive individuals develop faster and are therefore at an advantage in the laboratory. That entering diapause in the first generation is selectively disadvantageous in the field can be inferred from experimental data obtained the previous year, 1977, in the field cage where 650 F_1 pupae were recovered. These pupae were returned to field cage and not a single fly emerged in the spring of 1978.

The observed increase in diapause sensitivity following laboratory rearing has not been observed previously (Hoy, pers. comm.). In fact, the diapause response generally becomes less sensitive after many generations of laboratory rearing. In related experiments, selection for non-diapause in *D. antiqua* has been successful in five generations (van Heemert, pers. comm.), and crosses between the non-diapause strain and the normal laboratory strain (sensitive) indicate a multigenic mode of inheritance notwithstanding the rapid response to selection observed. This mode of inheritance has also been found in many other insect species, for example in *Diabrotica virgifera* LeConte (Branson, 1976; Krysan & Branson, 1977), *Heliothis zea* (Boddie) (Herzog & Phillips, 1974), *Pectinophora gossypiella* (Saunders) (Barry & Adkisson, 1966) and *Lucilia caesar* (L.) (Ring, 1971). For a comprehensive review of the genetics of diapause inheritance see Hoy (1978). The probable multigenic inheritance of diapause sensitivity in *Delia antiqua* makes the rapid change in diapause sensitivity following a minimum of six generations of laboratory rearing without conscious selection rather difficult to explain. However, subsequent experiments (Herfst, unpublished) under laboratory conditions (18°C and LD 8:16) that should induce diapause in about 50% of individuals (Loosjes, 1976), again resulted in 100% diapause in the laboratory strain. It can therefore be concluded that through unconscious selection during laboratory rearing without diapause induction, the diapause response of the laboratory strain has been significantly altered.

Interference with the life-cycle of insect pests through manipulation of the diapause response has been suggested as a means of control (Klassen et al., 1970; Foster & Whitten, 1974), and photoperiod manipulation by extending the daylength in the field with artificial light led to a decrease in the numbers of pupae overwintering in *Ostrinia nubilalis* (Hübner) and *Cydia pomonella* (L.) (Hayes et al., 1970). Conversely, in the present case, increased sensitivity to diapause prevented the operation of another method of control, namely use of translocations.

It is concluded that a control method using translocations in *D. antiqua* has to address itself to the diapause response of the released strains and that either by

laboratory selection or backcrossing to field individuals the correct diapause response has to be attained. This report emphasises the importance of quality when pest control techniques using translocations are being considered, and its lends support to the data already published on the significant differences between the laboratory strains and field strains in *D. antiqua* (Zurlini & Robinson, 1978; Robinson & Zurlini, 1979).

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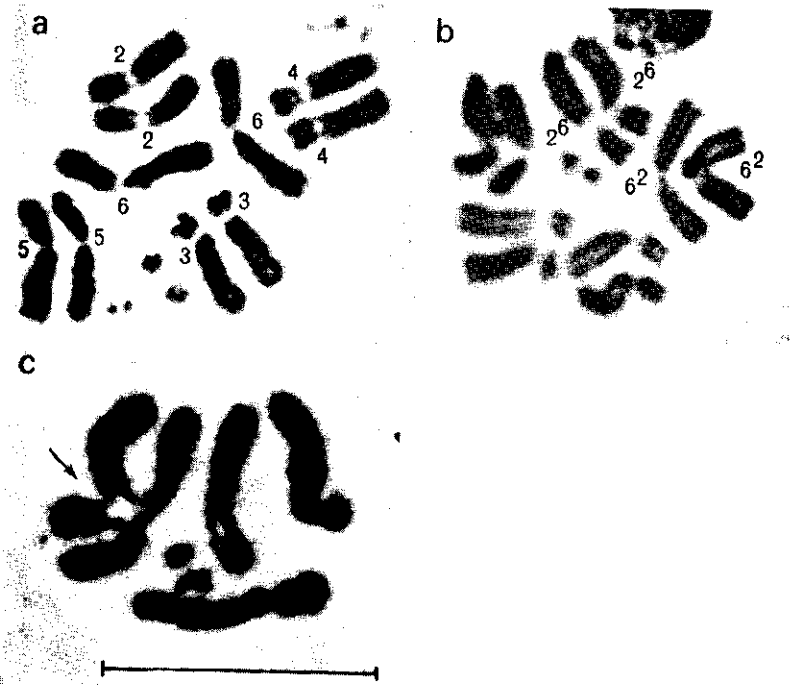

Karyotypes of *D. antiqua*. *a*, standard strain (+/+); *b*, translocation homozygote (*T*/*T*); *c*, translocation heterozygote (*T*+), translocation quadrivalent indicated with arrow. (Bar represents 10 μm.)

GENERAL DISCUSSION AND CONCLUSIONS

Sex ratio distortion

From the experiments reported in the first two chapters it could be concluded that in a native Dutch population of *H.antiqua* two types of males, XY_1 and XXY_2, coexist. Another very relevant conclusion is that the occurrence of some highly distorted sex ratios in favour of females as well as males, is caused by an unstable somatic behaviour of chromosome Y_2. Unfortunately, this unique system of sex ratio distortion is not suited for genetic control because an effective selection of predominantly male-producing male parents is impossible. The enhancement in frequency of XXY_2Y_2 karyotypes observed in progenies of XXY_2Y_2 males is too low to make use of it. It was surprising to find such a high frequency of XXY_2 males compared to XY_1 males, since it would be expected that the unstable somatic behaviour of Y_2 would be a disadvantage. However, a numerical variation of Y_2 has apparently no severe fitness consequences with the exception of a very low frequency of certain gynandromorphous types (chapter 1).

The discovery of a chromosome morphologically identical to Y_2 (indicated here as Y_m) in some females of a certain cross was unexpected since in all other crosses a strictly holandric inheritance of chromosome Y_2 had been observed. It was decided to study the inheritance of this chromosome, firstly, in order to test the verity of the hypothesis concerning sex-determination in *H. antiqua* and, secondly, to determine if male suppressing genes were perhaps present in the Y_m-carrying females. This last situation might be exploited to manipulate the sex ratio (chapter 2). The results of the various crosses demonstrated that chromosome Y_m was nothing more than a (mutated) non-functional Y_2 chromosome.

A promising method to produce only male progeny is selective killing of females using a Y-autosome translocation and a conditional lethal gene located on the translocated autosome. In *H.antiqua* as in other Diptera, the absence of crossing-over in males means that the conditional lethal locus remaining linked to the Y chromosome irrespective of its position relative to the translocation breakpoint. If a conditional lethal gene could be found on chromosome 2, T61 would be a very useful translocation for this purpose because of the low frequency of duplication/deficiency gametes and hence high fertility of this translocation; efficient mass rearing is therefore possible. In chapter 4 it was shown that also loci positioned on chromosome 6 can be exploited for genetic sexing if one

would make use of T14/T61 double-heterozygous males and T14-homozygous females. In comparison to the T61-heterozygotes, however, about twice as many eggs would be required in order to get the same number of males, since the double-heterozygotes produce some 60%-65% duplication/deficiency gametes.

Segregation

The most relevant results were obtained for translocations T14 and T61 (Chapters 3, 4 and 5). T14+ females showed highly aberrant adjacent 1 and 2 frequencies in comparison to T14+ males as well as to other translocations. The presence of very short interstitial segments, leading to short "Coorientation Determining Distances" (CDD's) between non-homologous centromeres, is suggested as the reason why a high frequency (25%) of adjacent 2 has been observed only in T14+ females (chapter 5). In T14+ males no adjacent 2 was found, attributable to the chromosome association. In males, until metaphase I, a very intimate meiotic pairing without chiasmata occurs which most probably results in very short CDD's between homologous centromeres thus favouring alternate and adjacent 1 orientations. The remaining translocations mentioned in chapter 5 all had longer interstitial segments which explains the absence or low frequency of adjacent 2 in both males and females.

The unusually high percentage (about 90%) of alternate observed in T61(2-Y) heterozygous males has been attributed to exeptional chromosome association. While in males heterozygous for any of the autosomal translocations (chapter 5) ring quadrivalents were always found at diakinesis, in T61 heterozygotes chain quadrivalents (with X and Y_2 in terminal position) were almost exclusively present. Due to these chain quadrivalents adjacent 1 was supposed to be unstable as in this orientation both X and Y_2 do not have a neighbouring centromere in trans-position (no counter-force for the pull exerted by the spindle fibres). Also, the very low frequency of adjacent 2 observed, might be caused either by a preferential coorientation of homologous centromeres (see above) or by "the unbalanced distribution of the chromosomes with respect to the equator" (see chapter 5).

From the segregation of the chain hexavalents in T14/T61 double-heterozygotes it was concluded that the stability (and therefore the frequencies) of the different orientation types depends on the relative positions of the centromeres.

Practical application of translocations in insect control

For maximum efficiency in the use of translocations for insect control it is necessary that homozygotes are as competitive as the wild type flies. However, for radiation-induced translocations this appears to be a major stumbling-block. In many insects it has been demonstrated that translocations are usually lethal or have reduced fitness as homozygotes, which is attributed to position effects, breakpoint damage or linked recessive lethals (Robinson, 1976). However, the results of some authors perhaps give too negative a picture as the methods used to isolate the homozygotes were not optimal (chapter 6). In addition, it is remarkable that many authors do not make allowance for possible negative effects of inbreeding. In a preliminary experiment it was shown that two generations of full-sib mating in the onion fly can already result in high larval mortalities and reduced pupal size. Therefore, special attention has been paid to this in the production of homozygotes. The T14-homozygotes used for the competition experiments had an average inbreeding coefficient of only 0.04. In how far the high fitness of the T14-homozygotes (measured under laboratory conditions) is due to the method used for the isolation of the homozygotes is difficult to say because too few translocations were tested.

The high sensitivity to diapauze of the T14-homozygotes found in the field-cage experiment demonstrates that data obtained in the laboratory cannot directly be extrapolated to the field situation (chapter 7). They also emphasize the importance of the "quality" of laboratory populations what are to be used for release.

The reproduction factor of *H.antiqua* under normal onion growing conditions is about 7 in the first and 3 in the second flight (Loosjes, 1976). Therefore, assuming density dependant factors to be absent, an overall population mortality of about 80% is needed to stabilize the population size. By the use of translocations alone, such on overall mortality would be difficult to realize. Assuming the fertility of a single heterozygote to be 50%, at least three different stocks of translocation homozygotes each showing a good fitness would seem to be necessary. Apart from the question if three such translocations could be isolated, a major problem will be how to get and particularly how to maintain an optimal ratio of the various (at least 27) karyotypes in the target population. Because of unstable equilibria (see below) there is a great risk that within a rather short time one or even more translocations disappear.

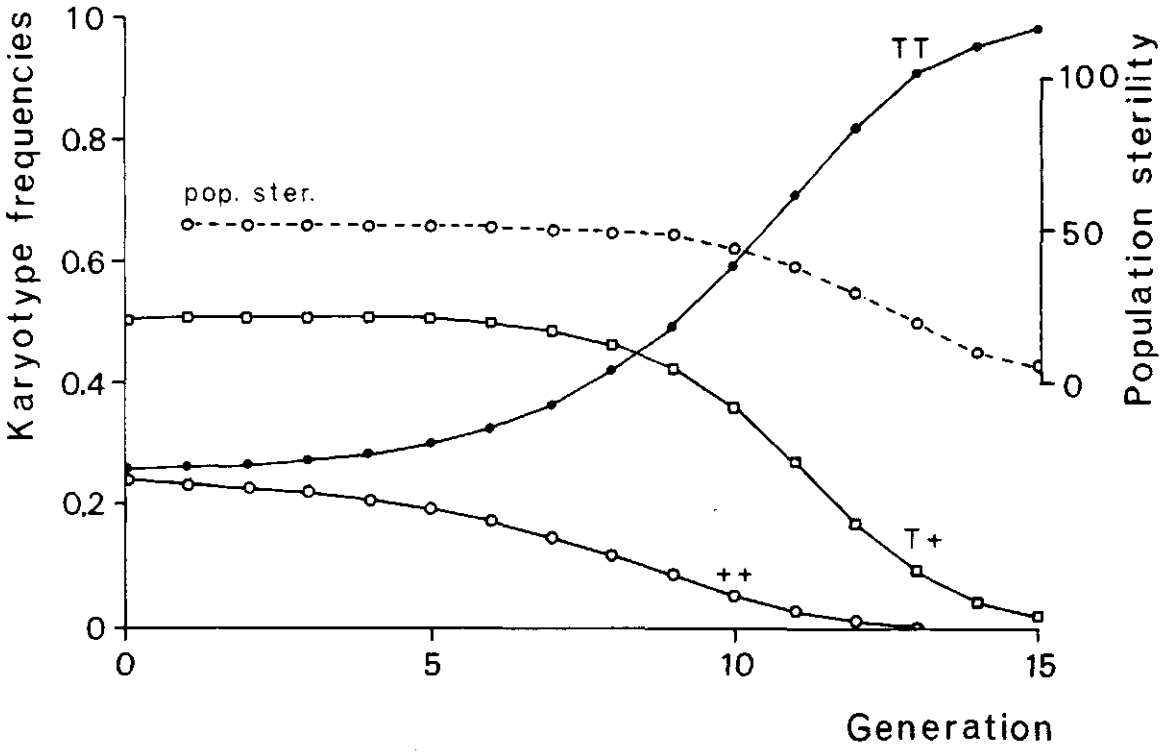

Fig. 1. Expected karyotype frequencies and population sterility (%) in 15 successive generations starting from a mixed population of TT,T+ and ++ with relative frequencies of 0.26, 0.50 and 0.24. Assumptions: (i) equal fitness of ++ and TT (ii) T+ produces 50% duplication/deficiency gametes (iii) maximum complementation and (iv) random mating. (v) absence of density-dependant factors.

Particularly when local differences in population size and karyotype frequencies occur, a monotoring of the populations followed by additional releases of certain karyotypes does not seem practicable.

A combined use of the translocation and sterile insect release method (SIRM) seems to offer better perspectives. The proposal is to introduce a translocation into a target population to obtain a certain reduction in fertility, followed by releases of sterile males (in subsequent generations) to "drag down" the sterility to a level required for a stabilization of the population at a low level. Using this method much fewer sterile males need be released. In the absence of a translocation the effective number of sterile males must be at least four times as high as the number of wildtype males to get an overall sterility of 80%. When, however, due to the presence of a translocation the population fertility is reduced by 40% it is sufficient to release only half that number of sterile males; since in a mixed population of ++, T+ and TT an unstable equilibrium will usually exist, a crucial point is the number of generations a level of sterility of 40% could be maintained. In Fig. 1 the expected karyotype frequencies and sterility levels are shown when the initial frequencies of ++, T+ and TT were 0.24, 0.50 and 0.26 respectively. Further assumptions are (i) the heterozygotes produce 50% duplication/deficiency gametes (ii) no differences in fitness between the homozygous karyotypes (iii) random mating and (iv) absence of density-dependant factors. Under these conditions an overall mortality of 40-42% can be maintained for about twelve generations (six years) but afterwards the population fertility increases rapidly. From an economical view-point a period of six years would be certainly attractive taking into account that at each generation only half the number of sterile males are needed. It should be realized, however, that the many other factors in the field conditions would have important consequences for this approach.

Many authors (see Robinson, 1976 and Feldmann, 1979) have suggested using translocations for gene frequency manipulation either to replace a population by a harmles type (refractory genes) or population suppression (conditional lethal genes). Many doubts exist, however, concerning the applicability of this method to the onion fly as well as to many other insects, especially if use was made of conditional lethality. To replace a population by translocation homozygotes of a certain desired genotype, a precondition is that the gene in question is very tightly linked to a suitable translocation. A "suitable" translocation infers that the homozygotes must be viable and show

Table 1. Egg-hatch and pupation percentages of single and double translocation heterozygotes (males and females). The single heterozygotes come from progenies of irradiated T14-homozygotes ($2^6 2^6 6^2 6^2$); the corresponding double heterozygotes were obtained after crossing with ++. Note: always 2^6 or 6^2 are involved in the translocations.

Trans-location	Trans-located chrom.[a]	Single heterozygotes			Double heterozygotes		
		% Egg-hatch	% Pu-pation	Number of eggs[b]	% Egg-hatch	% Pu-pation	Number of eggs
DT_1	$2^{6,L}\ 4^S$	70.4	44.1	1044(10)	52.8	27.1	1311(9)
DT_2	$2^{6,L}\ 4^L$	55.1	24.6	541(6)	52.2	23.3	1347(6)
DT_3	$2^{6,L}\ 4^S$ ♀	71.4	22.5	839(5)	51.9	15.7	846(8)
	+ inversion ♂	71.2	43.8	468(5)	47.9	31.5	620(4)
DT_4	$6^{2,S}\ 4^L$	75.1	39.1	902(10)	52.2	23.2	1071(10)
DT_5	$6^{2,S}\ 4^L$	82.5	48.7	1122(10)	61.1	32.7	1695(22)
DT_6	$6^{2,S}\ 5^L$	65.1	30.6	932(13)	43.3	27.4	961(10)
DT_7	$6^{2.S}\ 5^S$	95.3	51.7	611(5)	52.1	32.0	2176(10)
DT_{11}	$2^{6,S}\ 5^L$	70.9	44.6	2306(20)	38.8	21.6	1362(16)
DT_{14}	$6^2,5^S,3^L$	56.4	27.5	1261(11)	-	-	-
DT_{15}	$2^6,6^2,5^{L,S}$	35.6	17.2	1935(14)	35.0	20.7	1268(12)
DT_{16}	$2^6,6^2,3,4$	47.0	19.6	4120(26)	32.1	11.8	1139(12)

[a] L= long arm S= short arm

[b] The number of crosses are given in parentheses

good fitness. A large amount of screening will be necessary to find such a gene-translocation combination and it is very questionable if such an investment is justified as long as there is a risk that either by mutation or infrequent recombination "resistant" translocation homozygotes arise. For the onion fly another problem is that normally only two generations per year occur. Since in two generations a population replacement is impossible, one cannot make use of genes which for instance affect diapauze or cause lethality under a high or a low temperature condition. It should be noted that population replacement based on refractory genes has better perspectives because in contrast to conditional lethality, translocation homozygotes carrying the wild type alleles (which may arise by infrequent recombination or mutation) are not expected to have an advantage.

Multiple translocations

Following the irradiation of T14-homozygotes, several new translocations were isolated. Those translocations involving either chromosome 2^6 or 6^2 (the translocated chromosomes of T14) were selected for further studies. These new translocations behave as single reciprocal translocations as long as they are kept in the T14-homozygous background, but when crossed with ++ three-chromosome double heterozygotes are produced. In Table 1 the egg-hatch and % pupation are given for eleven such translocations. The main purpose of this study was to find out if perhaps a multiple translocation could be isolated which met the requirements for a stable equilibrium. Stam (1979) demonstrated that such a stable equilibrium can be expected for single reciprocal translocations if, firstly, the percentage of duplication/deficiency gametes produced by the heterozygotes is at least 80% which is unusually high for a single translocation; secondly, sufficient complementation occurs and, thirdly, the fitness of the homozygotes is about equal to ++ individuals. A specific condition for three-chromosome double-translocations is absence of crossing-over in the differential segment of the double-heterozygotes, otherwise undesirable recombinant karyotypes will arise. Since three-chromosome double-translocations tend to have low fertilities, we hoped to find one meeting these conditions. The results obtained for the double-heterozygotes (Table 1) were not very promising as none three-chromosome double-heterozygote produced more than 80% duplication/deficiency gametes. The frequencies of the balanced and unbalanced gametes

can be roughly estimated from the pupation percentages. Since in controls on the average about 80% of the larvae pupated, the pupation percentages of the double-heterozygotes have to be multiplied with a factor of 1.25 to get the frequency of balanced gametes. After this correction the frequencies of duplication/deficiency gametes for the first eight double-heterozygotes of Table 1 are estimated to be 60-75%(100 - pupation percentage x 1.25). The DT16 four-chromosome double-heterozygotes produced about 85% duplication/deficiency gametes.

The frequencies of unbalanced gametes were also cytologically determined (unpublished data). In testcrosses of male and female DT_{I} double-heterozygotes, 66% (n=315) and 69% (n=122) respectively of the eggs were unbalanced which corresponds very well with the 66% (100-27.1x1.25) estimated from the pupation percentages. In testcrosses of DT_{II} double-heterozygous males, 74% (n=217) of eggs were unbalanced, while the frequency estimated from the pupation percentage was 73 (100-21.6x1.25).

As mentioned earlier, another prerequisite for a stable equilibrium is, the absence of crossing-over in the differential segment of the double-heterozygotes (Note: males are achiasmate). However, in testcrosses of DT_{I} double-heterozygous females, despite a very short differential segment (see paper 6 for the breakpoint positions) a high recombination percentage of 7-8 was found. For DT_{II} double-heterozygous females, a much higher (42%) percentage was established but this is not surprising considering the much greater length of the differential segment.

A third very important factor for a stable equilibrium is the degree of complementation. On the basis of the karyotype frequencies observed in testcrosses of DT_{I} double-heterozygotes, only a moderate level of complementation was evident. If the recombinant karyotypes are excluded, in intercrosses of translocation double-heterozygotes ($T'+ \times T'+$) a ratio of ++ : $T'+$: $T'T'$ of 1 : 4.6 : 1 is expected while with a maximum complementation this ratio would be 1 : 10.4 : 1. For translocation DT_{II} highly significant differences in meiotic segregation between males and females have been observed and therefore only a very low level of complementation is expected.

The viability of three different double-translocation homozygotes ($T'T'$) has also been assessed. Two (DT_{I} and DT_{II}) appeared to be viable in the adult stage; DT_{5} homozygotes were inviable.

From these results it can be concluded that the chance of finding

a suitable translocation which meets the requirements for a stable equilibrium is very low. The only translocation which produced more than 80% duplication/deficiency gametes was DT_{16}. Since, however, in this translocation four chromosomes are involved it is almost certain that too little complementation will occur. Further, high percentages of recombinant karyotypes are expected and the chance that the homozygotes are viable seems very low (six new translocation breakpoints).

In many insects the"Sterile Insect Release Method"is not applicable because the radiation-sterilized individuals show a reduced competitiveness. In these cases one might consider to release low fertile, multiple translocation heterozygotes. Such heterozygotes can be produced by intercrossing of two different strains of (double-) translocation homozygotes. For the onion fly this method is not attractive because, firstly, the radiation-sterilized males show a good mating competitiveness (Loosjes, 1976) and, secondly, a sexing on large scale (necessary for the intercrosses) would be impracticable.

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SUMMARY

Experiments on sex determination of the onion fly, *Hylemya antiqua* (Meigen), are reported in the first two chapters. The main purpose was to establish if the sex ratio distortion observed in certain crosses, was based on a mechanism which could be used for genetic purposes. Two types of males, XY_1 and XXY_2, were found and the aberrant sex ratios appeared only to occur in progenies of XXY_2 (and some XXY_2Y_2) males. A numerical variation for Y_2 was frequently observed in embryos, larval ganglion cells and especially in testes, which is caused by numerical non-disjunction in somatic cells. The sex ratio distortion is attributed to such a numerical variation of Y_2 between primordial germ cells. Some progenies with a highly distorted sex ratio in male direction descended probably from XXY_2Y_2 males. Since an effective selection of XXY_2Y_2 males is not possible, this type of sex ratio distortion is not suited for application in a genetic control method. Other subjects treated in chapters 1 and 2 are gynandromorphism, a polymorphism with respect to the length of the X-chromosome and X-polysomy. In the progeny of one cross a chromosome morphologically identical to Y_2 but lacking its holandric inheritance was found. From its inheritance in various crosses it could be concluded that it was nothing more than a (mutated) non-functional Y_2 chromosome.

The meiotic segregation of a Y_1-linked translocation T61 is reported in the third chapter. The high percentage of alternate segregation observed in T61-heterozygous males ($22^{Y}XY^{2}$) was in good accordance with the high fertility of this translocation (95%). Because of an early separation (or asynapsis) of the centromeric regions of the acrocentric X and Y^2, chain quadrivalents with X and Y^2 in terminal position predominated in diakinesis/prometaphase I stages. In these chain quadrivalents adjacent 1 is supposed to be unstable since neither X nor Y^2 have a neighbouring centromere in trans-position. The very low frequency of adjacent 2 is attributed to a preferential coorientation of homologous centromeres. The suitability of T61 for genetic sexing (preferential killing of females) is discussed.

Double-translocation heterozygous T14/T61 males ($2^{6}2^{Y}6^{2}6XY^{2}$) were produced by intercrossing T14-homozygous females ($2^{6}2^{6}6^{2}6^{2}XX$) and T61-heterozygous males ($22^{Y}66XY^{2}$). These double heterozygotes showed several segregation types but for predominated. The total frequency of duplication/deficiency gametes was 60-65%. Application possibilities for these heterozygotes in genetic control of the onion fly are discussed.

The meiotic segregation of five different autosomal reciprocal translocations are given in chapter 5. For translocation T14, which has very short interstitial segments, significant differences in segregation between the

sexes were found. In males the ratio of alt.: adj.1:adj.2 was about 7:3:0 and in females 8:1:3. It is assumed that the very intimate meiotic pairing in males (without chiasmata) results in relatively very short "Coorientation Determining Distances" (CDD's) between the homologous centromeres. Due to these short CDD's a preferential coorientation between homologous centromeres is expected favouring alternate and adjacent 1 segregation. On the contrary, in females chiasmata act very probably as reference points for coorientation. Due to variable positions of the centromeres, in T14-heterozygous females less pronounced differences in CDD's between homologous and non-homologous are expected as in males. This is suggested to be the reason that in females a coorientation between non-homologous centromeres can occur resulting in a certain frequency of adjacent 2. The very low frequencies or absence of adjacent 2 in the other four translocations is attributed to their longer interstitial segments.

For translocation T14 a pure breeding stock of homozygotes could be obtained using egg-hatch percentages and cytology for the recognition of the karyotypes (chapter 6). In cage experiments under laboratory conditions the competitiveness of the translocation homozygotes was tested. Starting from an initial population consisting of equal numbers of translocation homozygotes (TT) and wild type flies (++), the karyotype frequencies were determined for three successive, non-overlapping generations. The results obtained did not suggest substantial differences in fitness between TT and ++.
In field cage experiments (chapter 7) a high diapause sensitivity was found for the translocation homozygotes as well as for the laboratory strain of wild type flies (++) used. As a consequence of this high diapause sensitivity the fitness of the homozygotes could not be determined under field conditions.

In the "General Discussion" different application possibilities of translocations for genetic control of the onion fly are discussed. A summary of unpublished data concerning the egg-hatch and pupal survival of eight three-chromosome double-translocation heterozygotes and three more complex translocation heterozygotes is also presented there. The aim of these experiments was to determine if perhaps a double translocation could be isolated meeting the conditions for a stable equilibrium. However, neither of these translocations met these conditions. Firstly, the frequencies of duplication/deficiency gametes were not high enough; secondly, insufficient complementation occurred and, thirdly, the recombination percentages in the differential segments were too high (resulting in undesirable karyotypes). Two of three double translocations tested, appeared to be viable as homozygotes in the adult stage.

SAMENVATTING

Het constateren, in bepaalde kruisingen van sex ratio's die significant afwijkend waren van 1:1 (Tabel 1, hoofdstuk 2) was de reden om de geslachtsbepaling bij de uievlieg nader te analyseren (hoofdstuk 1 en 2). Het primaire doel was te bepalen of het mechanisme ten grondslag liggend aan deze afwijkende sex ratio's, geschikt was voor gebruik in een genetisch bestrijdingsprogramma. Op grond van de cytologische waarnemingen is geconcludeerd dat twee typen mannetjes, XY_1 en XXY_2, coëxisteren in een uit Zeeland afkomstige populatie. Bovendien is een polymorphisme voor wat betreft de lengte van het X-chromosoom gevonden; in het lange acrocentrische X-chromosoom (X_1) was een intercalair proximaal segment aanwezig dat ontbrak in het normale korte, eveneens acrocentrische, X-chromosoom (X_s). Chromosoom Y_1 is homoloog met en morfologisch gelijk aan X_s. Daarentegen heeft het metacentrische Y_2 chromosoom geen homologe paringspartner, tenzij dit chromosoom in meervoud aanwezig is. In 22 van de 79 geanalyseerde kruisingen met XXY_2 en enkele XXY_2Y_2 vaders kwamen significant van de 1:1 verhouding afwijkende sex ratio's voor. Vooral in testes maar ook in embryo's en larvale ganglion-cellen is frekwent een numerieke variatie voor Y_2 vastgesteld; deze variatie wordt veroorzaakt door een somatische nondisjunctie van Y_2. Een groot deel van de afwijkende sex ratio's is waarschijnlijk een gevolg van een numerieke variatie voor Y_2 tussen de primordiale, germinale cellen. Enkele nakomelingschappen met een grote overmaat aan ♂♂ waren waarschijnlijk afkomstig van een kruising met een XXY_2Y_2 vader. Op grond van de waargenomen sex ratio's is geconcludeerd dat minimaal 3 primordiale cellen bij de vorming van de mannelijke germinale cellen betrokken zijn. Aangezien een hoge frekwentie (83%, n=24) van XXY_2 (Y_2) ♂♂ in vergelijking met XY_1 ♂♂ in de uit Zeeland afkomstige populatie is geconstateerd, lijkt het waarschijnlijk dat een numerieke variatie voor Y_2 niet of nauwelijks van invloed is op de fitness van de ♂♂. Weliswaar kunnen niet-reproducerende gynadromorfen ontstaan maar de frekwentie van deze typen is zeer laag (hoofdstuk 1). Een belangrijke conclusie van deze experimenten is, dat het mechanisme verantwoordelijk voor de sex ratio verstoring, ongeschikt is om te gebruiken voor een genetische bestrijding van de uievlieg. Een (efficiente) selectie van XXY_2Y_2, waardoor een grote overmaat van ♂♂ gekweekt zou kunnen worden, is niet mogelijk omdat deze ♂♂ niet fenotypisch te herkennen zijn en bovendien de frekwentie te laag is. In één kruising en nakomelingschappen hiervan is een chromosoom (Y_m) gevonden, dat morfologisch identiek was aan Y_2 maar geen holandrische vererving bleek te vertonen. De resultaten van een aantal kruisingsproeven wezen uit dat het

betreffende Y_m chromosoom een (gemuteerd) niet functioneel Y_2 chromosoom was.

De meiotische segregatie van een Y_1-gekoppelde* translocatie (T61) is vermeld in hoofdstuk 3. Het hoge percentage alternate (± 90%) waargenomen in de T61-heterozygote mannetjes correspondeerde goed met de hoge fertiliteit (± 95%). Waarschijnlijk door een vroege dissociatie (of asynapsis) van de centromeergedeelten van de acrocentrische X- en (translocatie) Y^2-chromosomen, bleken hoofdzakelijk ketenvormige quadrivalenten met X en Y^2 is distale positie in diakinese/prometaphase I stadia voor te komen. Het lage percentage adjacent 1 is waarschijnlijk een gevolg hiervan omdat in deze orientatie noch X noch Y^2 een aangrenzend chromosoom bezitten waarvan het centromeer gericht is naar de tegenovergestelde pool. Een voor een stabiele orientatie noodzakelijk geachte tegenkracht, voor de kracht uitgeoefend door de trekkende spoeldraden, ontbreekt in dit geval voor X en Y^2. De zeer lage frekwentie van adjacent 2 wordt waarschijnlijk veroorzaakt door een preferentiële coörientatie van homologe centromeren (zie hieronder). De geschiktheid van translocatie T61 voor een genetische "sexing" (preferentiële doding van ♀♀) is besproken.

Dubbel translocatie-heterozygote T14/T61 ♂♂ ($2^6 2^Y 6^2 6XY^2$) zijn verkegen door T61-heterozygote ♂♂ ($22^Y 66XY^2$) te kruisen met T14-homozygote ♀♀ ($2^6 2^6 6^2 6^2 XX$) (hoofdstuk 4). Voor deze T14/T61 heterozygote ♂♂ is een hypothetische relatie vastgesteld tussen de frekwenties van een aantal orientatie-typen en de relatieve posities van de centromeren in de betreffende orientaties. Het percentage duplicatie/deficientie karyotypen, waargenomen in jonge embryo's van toetskruisingen, bleek goed te corresponderen met de mortaliteit in laat embryonale en larvale stadia. Een klein percentage ongebalanceerde karyotypen werd gevonden in pupale en adulte stadia.

Een opmerkelijk segregatiegedrag is geconstateerd voor translocatie T14 (hoofdstuk 5). In T14+ ♂♂ was de ratio van alt.: adj.1 :adj.2 7:3:0 terwijl in overeenkomstige ♀♀ deze verhouding 7:1:3 was. De afwezigheid van adjacent 2 in ♂♂ is waarschijnlijk te wijten aan de sterke meiotische paring (zonder chiasmata) in deze sexe. De homologe centromeren worden verondersteld zeer nauw met elkaar geassocieerd te zijn, resulterend in zeer korte "Coorientation Determining Distances" (CDD's). Als gevolg hiervan wordt, ondanks zeer korte interstitiële segmenten van T14, een preferentiële coörientatie tussen homologe centromeren verwacht waardoor alleen alternate en adjacent 1

* in het volgende met Y aangeduid

orientaties ontstaan. In vrouwtjes, daarentegen zijn de posities van de chiasmata hoogstwaarschijnlijk bepalend voor de CDD's tussen de centromeren. Uitgaande van een variabele positie van de centromeren zullen in de translocatie-quadrivalenten van T14-heterozygote ♀♀ de verschillen in CDD's tussen homologe en niet-homologe centromeren veel minder geprononceerd zijn dan in de ♂♂, waardoor ook een coörientatie tussen niet-homologe centromeren kan optreden, resulterend in een bepaald percentage adjacent 2. De overige vier translocaties vermeld in hoofdstuk 5 hadden langere interstitiële segmenten, hetgeen de afwezigheid of lage frekwenties van adjacent 2 in zowel ♀♀ als ♂♂ verklaart.

De methode waarmee T14-translocatie-homozygoten, met lage inteeltcoefficienten, zijn geïsoleerd, staat vermeld in hoofdstuk 6. De betreffende methode is vergeleken met andere in de literatuur beschreven methodes. Om de competiviteit van de translocatie-homozygoten (TT) te vergelijken met wild-type vliegen (++) zijn kooiproeven onder laboratoriumomstandigheden verricht. De uitgangspopulatie bestond uit een gelijk aantal ++ en TT individuen (n=3000). In drie achtereenvolgende, niet overlappende generaties met telkens een populatiegrootte van 3500-4000 vliegen, zijn de karyotypefrekwenties bepaald. De verkregen resultaten suggereerden dat er nauwelijks of geen verschillen in competiviteit tussen TT en ++ individuen waren. In een afzonderlijk experiment zijn de eiproduktie en levensduur van 35 TT en 28 T+ ♀♀, afkomstig van vijf verschillende TT x T+ kruisingen, vergeleken. Voor slechts één kruising zijn significant hogere waarden voor de eiproduktie en levensduur voor de T+ ♀♀ geconstateerd. Ook in veldkooiproeven is getracht de relatieve fitness van de T14-homozygoten te bepalen (hoofdstuk 7). Uit deze experimenten is echter nauwelijks informatie verkregen, omdat vrijwel alle poppen (++,T+ en TT) van de eerste generatie in diapauze zijn gegaan. Mogelijke oorzaken van deze hoge diapauzegevoeligheid zijn aangegeven.

Een aantal complicaties en de perspectieven van het gebruik van translocaties voor een genetische bestrijding van de uievlieg staan vermeld in de "General Discussion and Conclusions". In dit hoofdstuk is ook een samenvatting gegeven van nog niet gepubliceerde resultaten betreffende de embryonale sterfte en pupale overleving van 11 dubbele translocatie-heterozygoten. Deze dubbele translocatie-heterozygoten zijn verkregen in nakomelingschappen van bestraalde T14-homozygoten, na kruising met ++. Het doel van deze proeven was te bepalen of het mogelijk zou zijn een dubbele translocatie (met één translocatie-

multivalent) te verkrijgen die zou voldoen aan de eisen voor een stabiel evenwicht. Uit de resultaten is echter gebleken dat het percentage duplicatie/deficientie gameten toch nog te laag is. Bovendien is uit de cytologische analyse van 2 dubbele translocaties, gebleken dat slechts een beperkte complementatie verwacht mag worden, te laag voor een stabiel evenwicht. Een derde probleem is dat door overkruising in het differentiële segment ongewenste recombinanten kunnen ontstaan; in één dubbele translocatie bleek ondanks een kort differentieel segment een hoog recombinatiepercentage (6-7%) voor te komen. Twee van de drie getoetste dubbele translocaties bleken als homozygoot levensvatbaar in het adulte stadium.

CURRICULUM VITAE

De schrijver werd geboren op 30 mei 1949 te Epe. Na het behalen van het einddiploma HBS-b in 1967 begon hij in dat zelfde jaar met zijn studie aan de Landbouwhogeschool te Wageningen. De studierichting was plantenveredeling met als keuzevakken erfelijkheidsleer (verzwaard) en algemene plantenziektekunde. Het doctoraalexamen werd afgelegd in januari 1975. Vanaf maart 1975 tot eind 1978 was hij als promotie-assistent, respectievelijk tijdelijk medewerker in dienst van de Landbouwhogeschool en het ITAL te Wageningen. Gedurende deze periode werd het hier gerapporteerde onderzoek verricht. Sinds augustus 1979 is hij werkzaam als leraar plantenveredeling en gewasbescherming op de RHLS te Deventer.