

# Karyotype displacement in a laboratory population of the two spotted spider mite *Tetranychus urticae* (Koch): Experiments and computer simulations

A. M. Feldmann<sup>1</sup> & M. W. Sabelis<sup>2</sup>

<sup>1</sup> Association Euratom-ITAL, P.O. Box 48, Wageningen, The Netherlands

<sup>2</sup> Department of Theoretical Production Ecology, Agricultural University, Wageningen, The Netherlands

## Abstract

Three different strains, homozygous for a radiation induced structural chromosome mutation (T), exhibiting negative heterosis, were tested for their ability to displace the standard (wild-type) karyotype from experimental populations. The experimental populations were initiated by mixing fertilized females of both a T strain and the standard strain at different ratios. Two of the T strains showed the ability to displace the standard karyotype if the initial frequency of the T karyotype was at least 0.65. The additional release of T males into the experimental population accelerated considerably the process of displacement of the standard karyotype, especially if the initial T karyotype frequency was 0.65.

A computer model for simulating the process of population displacement in *Tetranychus urticae* was developed. The model accounts for variation in developmental time and for the age dependency of variables related to fitness. The simulations showed a good correlation with the experimental results. A system analysis on the sensitivity of the model output to varying different population parameters demonstrated that especially the relative number of males produced by a T strain and the female developmental rate were of significant importance to the population displacement ability of a T strain. The negative influence of genetic markers on general fitness and various aspects of practical application of the method are discussed.

## Introduction

Some common and serious pests were successfully combatted by the general introduction of synthetic insecticides shortly after the Second World War. However, although some old pests declined, other pests, previously unknown as such became significant (upset pests, De Bach, 1974). One of these upset pests is *Tetranychus urticae* (Huffaker *et al.*, 1969, 1970; McMurtry *et al.*, 1979) which for two decades has been satisfactorily controlled by the development of a series of acaricides, just keeping ahead of the astonishing capacity of this species for development of resistance. The arsenal of acaricides is not depleted yet but past experience of unexpected resistances towards new chemicals, the awareness of the en-

vironmental impact of agricultural chemicals together with the rising costs of development and legal registration of new pesticides point to the need for the development of alternative control possibilities (Bravenboer, 1975; Chant, 1971; Helle, 1969).

As one of these alternative pest control strategies, it has been proposed to reduce the fertility of a mite population by the release of radiation sterilized males (Nelson, 1970; Nelson & Stafford, 1972) or by the release of males which carry chromosomal rearrangements (van Zon & Overmeer, 1972; Overmeer & van Zon, 1973). Control of pest populations of *Tetranychus urticae* by the release of radiation-sterilized males is unlikely to be successful because of a significant reduction in male mating competitiveness commencing two days after irradiation

(Feldmann, 1977). This is a serious drawback because for successful control released sterilized males must have a long term effect on population fertility, since greenhouse populations of *Tetranychus urticae* are multivoltine with overlapping generations.

Young males continually emerge in such populations and the ageing released males are required to compete effectively with them for mating with newly emerged females. Only a frequent overflooding of the pest population by irradiated, 1-day-old males, would ensure that a high frequency of females would be effectively inseminated by the irradiated males. Such a frequent release schedule would not be practical or economic. Thus it is worthwhile to consider methods having a long term effect, associated with a low release frequency of genetically altered individuals. The translocation method might fit this specification (Serebrovski, 1940; Curtis, 1968a; Laven, 1968; Robinson, 1976).

The maximal population suppressing effect by using single translocations is only about 50%, but this could be enhanced if multiple translocation stocks were used in a control program or if different translocation strains were successively released into a pest population. However, because of the high rate of natural increase ( $r_m$  per day = 0.273) (Feldmann, 1980; Watson, 1964) and the large number of generations per year under greenhouse conditions (20–30) it is felt that a genetic method relying on suppression of population growth by the introduction of partially sterilizing factors, would have only a very limited effect (Curtis, 1968a). A genetic method exploiting these biological properties of *Tetranychus urticae* and not subject to the effect of density-dependent regulation (Curtis, 1968a; Robinson, 1976) is the Population Displacement Technique based on negative heterosis (Curtis, 1968b; Whitten, 1970, 1971, Whitten & Foster, 1975; Fitz-Earle, 1975; Fitz-Earle *et al.*, 1973, 1975). The principle is to release into a wild-type population a karyotype, having intrastrain fertility, but a degree of interstrain sterility when mated to wild type, and to continue releases until the frequency of the released type exceeds the unstable equilibrium point (Li, 1978).

The wild type would then be completely displaced from the population by natural selection without any further releases. The velocity of the process of displacement depends on the relative

fitness of the strains, on the initial frequency established by the releases, the level of hybrid sterility and whether generations are discrete or overlapping. It is a condition for the success of this technique that no behavioural isolation barrier for cross mating exists or can rapidly evolve between the strains.

Displacement of a pest population by a population possessing certain beneficial traits has been considered, mainly theoretically, as a promising method for long term control of a pest population (Curtis, 1968b; Whitten, 1970). Examples of such beneficial traits are conditional lethal genes (e.g. sensitivity to high (summer) or low (winter) temperatures, sensitivity to insecticides) and genes causing vector refractoriness (e.g. in mosquitoes, changing them from malaria or filariasis transmitters to non-transmitters). Such beneficial genes require a genetical carrier mechanism for transporting and spreading them into a natural population. Meiotic drive (Hickey & Craig, 1966; Craig, 1963), cytoplasmic incompatibility and chromosomal translocations (Curtis, 1968b, 1979) respectively may provide such a transport mechanism but their value is largely determined by the relative fitness of the strains which could be released.

The isolation of temperature-sensitive mutations should be rather easy in this species, due to its arrhenotokous parthenogenetic mode of reproduction (Smith, 1977). Inseminated females produce both fertilized and unfertilized eggs. Non-inseminated females produce only unfertilized eggs. The diploid females develop from fertilized eggs and the haploid males develop from unfertilized eggs.

The aim of this paper is to report results of experiments demonstrating displacement of a wild-type karyotype of *T. urticae* by a radiation-induced rearranged karyotype in an experimental population and to verify by computer simulation the relative importance of various population parameters in the process of population displacement.

## Material and methods

### Biology

#### Strains and experimental conditions

A large population of the wild-type strain of *Tetranychus urticae* was kept in the laboratory for

six years, which corresponds with about 150 generations. Three strains, each homozygous for a different structural chromosome mutation, were used in separate experiments on population displacement. These homozygous structural chromosome mutation strains (referred to subsequently as T strains) were derived after appropriate inbreeding (van Zon & Overmeer, 1972) following induction of the chromosomal rearrangement by 200 rad (2 Gy) of X-rays, in mature sperm. The T strains had been kept in the laboratory since April 1975, which corresponds to about 39 generations until their use in the experiments and they are designed subsequently as T-2, T-11 and T-17.

Both the wild-type and the T strains were kept at  $28 \pm 2^\circ\text{C}$ ;  $60 \pm 10\%$  relative humidity; 12 000 lux for 16 hours in a cycle of 24 hours. They were maintained on whole bean plants (*Phaseolus vulgaris*) and at regular intervals when they became overcrowded, a proportion were moved to fresh plants.

For use in the experiments, the mites were taken from cultures on detached bean leaves, laid on tap-water soaked cotton wool. These mite cultures were started by collecting 16 mature egg-laying females from the culture on bean plants and placing them on the detached leaves. These females were allowed to produce eggs for 3 days, after which the females were discarded. Large numbers of female deutonymphs and female teleiochrysalids could be collected 7–10 days after removal of the parental females. Males were derived from identical cultures, started from unfertilized females, which were collected being in the last quiescent developmental stage.

Single-pair matings were performed on discs, from primary leaves of beans, of 1 cm diameter. In order to confine the mites to the leaf area, the discs were surrounded by thin walls of cotton, soaked with tap water. Twenty discs were placed on a layer of cotton soaked with tap water, in a plastic tray (10 × 10 × 3 cm).

#### *Design of the population displacement experiment*

Virgin females were collected from mite cultures of each of the strains, as quiescent deutonymphs (teleiochrysalid stage) and were put on a bean leaf disc (diameter 3 cm), the three strains being kept separately. Equal numbers of 1-day-old males, of the same karyotype, were added to the females. In

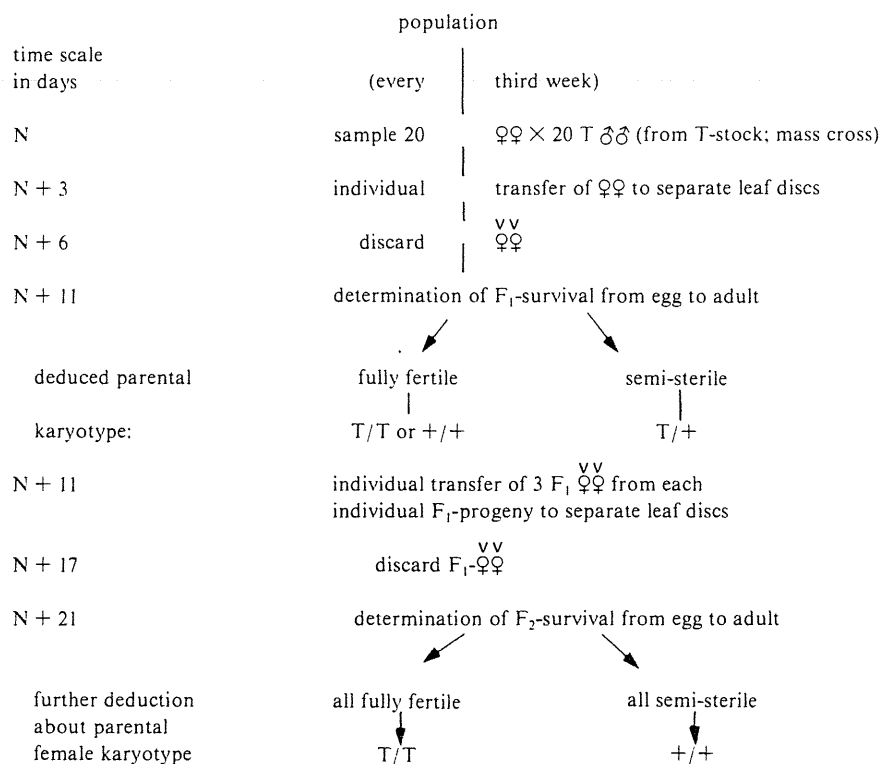
this way the virgin females were mated immediately after ecdysis with males of the own karyotype (homogamic mating). The displacement experiments were performed on three young bean plants, sown together in a pot of 10 cm diameter. The plants were used from the first leaf stage. The wild-type females together with females of one of the T strains were put on the bean plants at an adult age of 4 days. At that time the females have mated and produce both fertilized and unfertilized eggs. In this way both the wild-type females and the T strain females are physiologically synchronized, so that there is fair competition in the initiation of the population experiment. The initial ratios of females of the T strain to wild-type females were 10:10, 13:7 and 16:4 respectively. Each experiment was carried out in triplicate for each of the T strains.

In separate experiments, the influence on the process of population displacement of releasing T males into the experimental population was studied. The experimental design was as already described except that on the 7<sup>th</sup>, 9<sup>th</sup> and 11<sup>th</sup> day after placing the females on the plants, 100 males of the corresponding T strain were released on to each plant. On these days, the first ecdysing F<sub>1</sub> females were expected in the experimental populations so that the probability of insemination of these females by T males was increased appreciably, thus favouring fixation of the T karyotype. The population displacement experiments lasted several months and replacement of the bean plant, infested by the mite populations, was required about every second week. This was done by transferring three mite-infested leaves, taken at different heights from the old plant, to the fresh bean plant. In this way at least 1000 individuals, at all stages of development were used to start each new population.

#### *Determination of the T-karyotype frequency*

During the course of the population displacement experiments, the frequency of the T karyotype was assessed every third generation. The method of estimation of the relative frequencies of the karyotypes in a population was based on fertility measurements (Fig. 1).

The determination of the karyotype by fertility measurement is one of the major technical advantages of the arrhenotokous parthenogenetic mode of reproduction of *Tetranychus urticae* (Feldmann, 1979). Virgin females (teleiochrysalids), sampled



Data from the fertility estimation on days N + 11 and N + 27 used to estimate karyotype frequencies in the population on day N.

Fig. 1. Scheme for estimation of karyotype frequency among females, sampled as virgins from spider mite (*Tetranychus urticae*) experimental populations, consisting of a mixture of females, possessing either a rearranged (T) karyotype (homo- or heterozygous) or the standard (wild-type) karyotype. T/T: females homozygous for the T karyotype; +/+ : homozygous wild-type females; T/+ : heterozygous females.

from the experimental population are either homozygous for the structural chromosomal rearrangements (T/T), heterozygous (T/+) or wild-type (+/+). T/T and +/+ females are fully fertile while T/+ females are semi-sterile (Robinson, 1976).

T/T and +/+ females can be distinguished from each other by crossing the sampled virgin females to males of the appropriate T strain. T/T females give rise to fully fertile F<sub>1</sub> females while +/+ females produce, after being fertilized by T males, only semi-sterile (T/+) F<sub>1</sub> females. Each female collected from the population represents two sets of chromosomes; the frequency of the sets of chromosomes having the T karyotype is calculated on the total number of chromosome sets which could be characterized by the fertility tests. The actual procedure of karyotype frequency estimation was

as follows (Fig. 1): From each population at least 20 teliochrysalids (last quiescent developmental stage) from at least three different leaves per plant, were collected. The virgin females were set on a leaf disc together with equal numbers of males of the corresponding T strain for 2 days. This was done for each population separately. Subsequently, the females were singled on bean leaf discs (diameter 2 cm) for producing eggs during 3 days, after which the females were discarded. The percentage of survival from the egg stage till adulthood was determined for all the different F<sub>1</sub> progenies. From each progeny having full viability three F<sub>1</sub> females were collected. These F<sub>1</sub> females were singled on bean leaf discs for 5 days and discarded subsequently. The survival of the different F<sub>2</sub> progenies was determined. If all the three tested F<sub>1</sub> females,

produced by the same parental female, proved to be semi-sterile, then it was assumed that the parental female had been homozygous for the wild-type karyotype; it was assumed that the parental female had been homozygous for the chromosomal rearrangement if one or more  $F_1$  females were fully fertile.

### *Computer simulation*

#### *Input data for computer program simulating population displacement*

Life table data of both the wild-type strain and of T-2 and data on age-dependent male mating competitiveness (Feldmann, 1981) were used in a computer program, which was developed for simulating population growth and the process of karyotype displacement.

#### *System analysis*

The structure of the models and the underlying assumptions are explained in this section. The models are based on the assumption, that a structural chromosome mutation (T) behaves as a heritable unit which does not lose its integrity during the process of segregation of chromosomes in meiosis.

The relative importance of different parameters (sex ratio, male mating competitiveness, developmental rate, etc.), was determined by a check on the sensitivity of the model output (= relative frequency of T karyotypes) to a standard perturbation of the input values.

The experimental circumstances were chosen as a standard for all simulations. The explanatory value of the input data and the structure of the model were validated by comparison of the output with the experimental results.

#### *Models on karyotype displacement*

Assume an isolated greenhouse population of spider mites, containing wild-type karyotypes ( $+/+ \text{♀ en } + \text{♂}$ ), chromosome mutation homozygotes ( $T/T \text{ ♀♀ en } T \text{ ♂}$ ) and heterozygotes ( $T/+ \text{ ♀♀}$ ). Furthermore, the karyotypes occupy the same niche and the population size and biology allow for random mating among karyotypes. The first mating of the female determines the karyotype of the female progeny (Helle, 1967). Chromosome translocation heterozygotes ( $T/+$ ) are normally semi-

sterile (negative heterosis) in contrast with the homozygotes of both karyotypes. The relative frequency of the structural chromosome mutation in a population depends on its viability and the initial relative frequency of the T karyotypes. These parameters determine the probability of heterogametic matings ( $T/T \text{ ♀} \times + \text{♂}$ ;  $+/+ \text{ ♀} \times T \text{ ♂}$ ). If the probability of mating with a T ♂ is permanently greater than 50%, either due to T-male release or fitness, then the frequency of T karyotypes will exceed the point of unstable equilibrium (Curtis, 1968b) resulting in the fixation of the T karyotype.

The direction and the rate of the displacement process are analyzed by two types of deterministic models. In the first model discrete generations are assumed and age-dependent variables, such as reproduction etc., are treated as weighted means per generation; the relative frequencies of the karyotype in generation  $n + 1$  are computed from those of generation  $n$ ; the males of generation  $n$  do not interfere with the mating pool of generation  $n + 1$  and vice versa (discrete generations).

For spider mites the assumption of discrete generations is unrealistic, because the generations overlap, since breeding is continuous and the reproductive period is longer than the developmental time. Therefore a second model was constructed which accounts for overlapping generations and thus takes account of the age-dependent character of some variables and of variation in developmental time. Because the set up of the population experiment was such, that the age distribution was not stable during the whole period, analytical models are out of the question and numerical simulation models, accounting for non-stable age distributions, are needed. The short time intervals, needed to integrate the age-dependent rates, increase the consumption of computer time more than tenfold. However, in this model the actual number of mites in each developmental stage or adult age can be computed, so that the functional sex ratio (= males / (males + preoviposition ♀♀)) is monitored throughout the process of population growth. Moreover the numerical integration procedure enables the timed modelling of actions, such as male release, spraying, etc., destabilizing the age distribution so that analytical models are not adequate. By comparison of the above models it is possible to detect to what extent the process of population displacement is affected by the degree of

overlap of the spider mite generations, that is to say the degree of interference of males from generation  $n$  with those from generation  $n + 1$ .

### (1) Discrete generations

For successive distinct generations ( $n$ ) the relative frequencies ( $= f_n^k$ ,  $k$  = karyotype) of the karyotypes were calculated from the products of the mating probabilities with a T ♂ or a + ♂ ( $= p_n^T$  for T males and  $p_n^+$  for + males) and the karyotype frequencies of the females (T/T; T/+, +/+). In the case of random mating this probability is equal to the frequency of T (or +) males, relative to the total adult male population. The effect of mating competitiveness can be incorporated by multiplication of the karyotype frequency (or number) of the weakest competitor by a reduction factor ( $= c$ ) (see also Dietz in Pal & LaChance, 1974). In this representation, the competitive ability of a T male is equivalent to  $c$  percent of that of a + male:

$$p_n^T = c \cdot f_n^T / (c \cdot f_n^T + f_n^+)$$

$$p_n^+ = f_n^+ / (c \cdot f_n^T + f_n^+)$$

The net reproduction ( $R$ ) of T/T and T/+ females B and A respectively, was expressed relative to that of the +/+ females as follows:

$$B = \frac{R_{T/T}}{R_{+/+}} \text{ and } A = \frac{R_{T/+}}{R_{+/+}}$$

$R$  = net reproduction (male + female offspring)

$$R = \sum l_x \cdot (m_x + m'_x)$$

It is assumed that  $R_{T/+} = R_{+/+} \cdot (1 - hs)$  where  $hs$  = level of hybrid sterility. The sex ratio,  $S$ , (ratio of the number of females to the sum of females and males) was computed as a weighted mean over the age classes of the parental females:

$l_x$  = female survival probability to age  $x$

$m_x$  = reproduction at age  $x$  (female offspring)

$x$  = age in days

$m'_x$  = reproduction at age  $x$  (male offspring)

$$S = \frac{\sum l_x m_x}{\sum l_x \cdot (m_x + m'_x)}$$

(Symbols according to Birch, 1948).

The following set of equations computing karyotype frequencies in generation  $n + 1$  ( $f_{n+1}$ ) from those in the preceding generation ( $f_n$ ) can now be

derived for the case of the arrhenotokous parthenogenetically reproducing spider mite:

$$f_{n+1}^{T/T} = p_n^T \cdot (B \cdot f_n^{T/T} \cdot S^{T/T} + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot S^{T/+}) / f_n$$

$$f_{n+1}^{T/+} = \{ p_n^T \cdot (f_n^{+/+} \cdot S^{+/+} + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot S^{T/+}) + p_n^+ \cdot (B \cdot f_n^{T/T} \cdot S^{T/T} + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot S^{T/+}) \} / f_n$$

$$f_{n+1}^{+/+} = p_n^+ \cdot (f_n^{+/+} \cdot S^{+/+} + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot S^{T/+}) / f_n$$

$$f_{n+1}^T = \{ B \cdot f_n^{T/T} \cdot (1 - S^{T/T}) + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot (1 - S^{T/+}) \} / f_n$$

$$f_{n+1}^+ = \{ f_n^{+/+} \cdot (1 - S^{+/+}) + \frac{1}{2} \cdot A \cdot f_n^{T/+} \cdot (1 - S^{T/+}) \} / f_n$$

$$f_n = B \cdot f_n^{T/T} + A \cdot f_n^{T/+} + f_n^{+/+}$$

Given a set of initial values for  $f_0^k$  ( $k$  = karyotype), recursive use of these equations for a number of generations will show the trend of the displacement process.

The average generation time ( $G$ ) at 28 °C is 14–15 days, which is computed according to the following formula:

$$G = \frac{\sum x \cdot l_x \cdot m_x}{\sum l_x \cdot m_x}$$

### (2) Overlapping generations

The model for karyotype displacement over a number of discrete generations can be extended to include stage, and age-dependent variables such as developmental time, reproduction, mortality, sex ratio and male mating competitiveness. For this purpose the number of individuals must be monitored at any moment. The technique used is comparable to the principle of train movement, the rails being equivalent to the time axis. The stream of individuals developing from egg to maximum age is subdivided into development- or age classes (carriages), together forming a train. In principle the content of each carriage is transferred into the next after the elapse of the appropriate residence time. Dispersion in developmental time is simulated according to the fractional repeated shift method of Goudriaan (1973), which allows the mimicking of the measured standard deviations of the develop-

mental period.

Hence the number of mites per class is monitored, accounting for the inflow from the preceding carriage and the outflow to the following carriage. In addition the mortality is accounted for by a relative mortality rate ( $= (\ln(N_0) - \ln(N_t))/t$ ) ( $t$  = duration of developmental stage). All rate variables can be related to driving variables such as temperature or relative humidity. The newly laid eggs are transferred to the first carriage of the train, which completes the developmental cycle. In this way, the model simulates the growth processes of the different karyotypes, which results in overlapping generations. To each female age class of each karyotype is assigned its characteristic reproductive potential, sex ratio of the offspring and mortality and to each male age class is also assigned its inter- and intrakaryotypic mating competitiveness coefficient. In the model, five categories are distinguished: eggs, juveniles, males, preovopositional and ovopositional females. In the preovopositional phase three female karyotypes are partitioned among  $2 \times 3$  possible mating types depending on the proportion of T and + males in the adult male population and on mating competitiveness. Finally the karyotypes of the offspring depend on the mating combination of the parents, according to the formulae presented (1).

The simulation model is presented in the Appendix. The model was validated for the case of *Tetranychus urticae* on glasshouse roses as far as the quantitative aspects of population growth are concerned (Sabelis, in prep.).

## Results

### Population experiments

*Population experiments without releases of T males*  
Figure 2 gives a graphic representation of the percentage of the T-17, T-11, and T-2 karyotypes and the number of days elapsed from the initiation of the experiments. The effects of three different initial ratios, 10:10, 13:7 and 16:4 of T/T females to +/+ females respectively are demonstrated in Figure 2. The open circles, the crosses and black dots respectively represent the three replicate populations, at a certain time after initiation of the experiments. Figure 2 demonstrates the following

features:

(a) Initial release ratio of 10 T/T females to 10 +/+ females: None of the three karyotypes tested (T-11, T-17 and T-2) reached fixation at this ratio. A clear trend towards displacement of the karyotypes T-11 and T-2 by the + (wild-type) karyotype was observed. In one population an equilibrium existed between the + and T-17 karyotypes.

(b) Initial release ratio of 13 T/T females to 7 +/+ females: There was an increase of the T-17 karyotype to over 90% in two of the replicate populations, within 150 days after the initiation of the experiment. Of the populations involving strain T-2 a sample from one population taken 18 generations after initiation of the experiment contained only T/T females. The percentage of the T karyotype in the other populations of line T-2 oscillated around 50% in the successive generations in one population while in the other population a downward trend in the percentage of the T karyotype was observed.

In the populations involving strain T-11, no increase in the percentage of the karyotype was observed. In two populations the percentage of the T karyotype oscillated around 50% and in the other population a decrease in the percentage of the T karyotype occurred during successive generations.

(c) At an initial release ratio of 16 T/T females to 4 wild-type females it was noted that when fixation of T-2 took place, this process of fixation required about 110 days and it was faster than at the 13:7 release ratio. The process of fixation at the 16:4 release ratio also occupied about 110 days in one population involving strain T-17 and about 150 days in another while the third replicate showed a decline in T-17 frequencies.

### Population experiments involving release of males

The influence of supplementary male releases on the process of population displacement was studied with the T-2 strain. The percentages of the karyotype T-2 in the experimental populations in relation to the length of time after the initiation of the experiments, are represented in Figure 3. Days 7, 9 and 11, on each of which 100 T males were released in each experimental population, are marked on the horizontal axis of Figure 3. The dotted curves represent the course of the process of karyotype displacement without male release and correspond

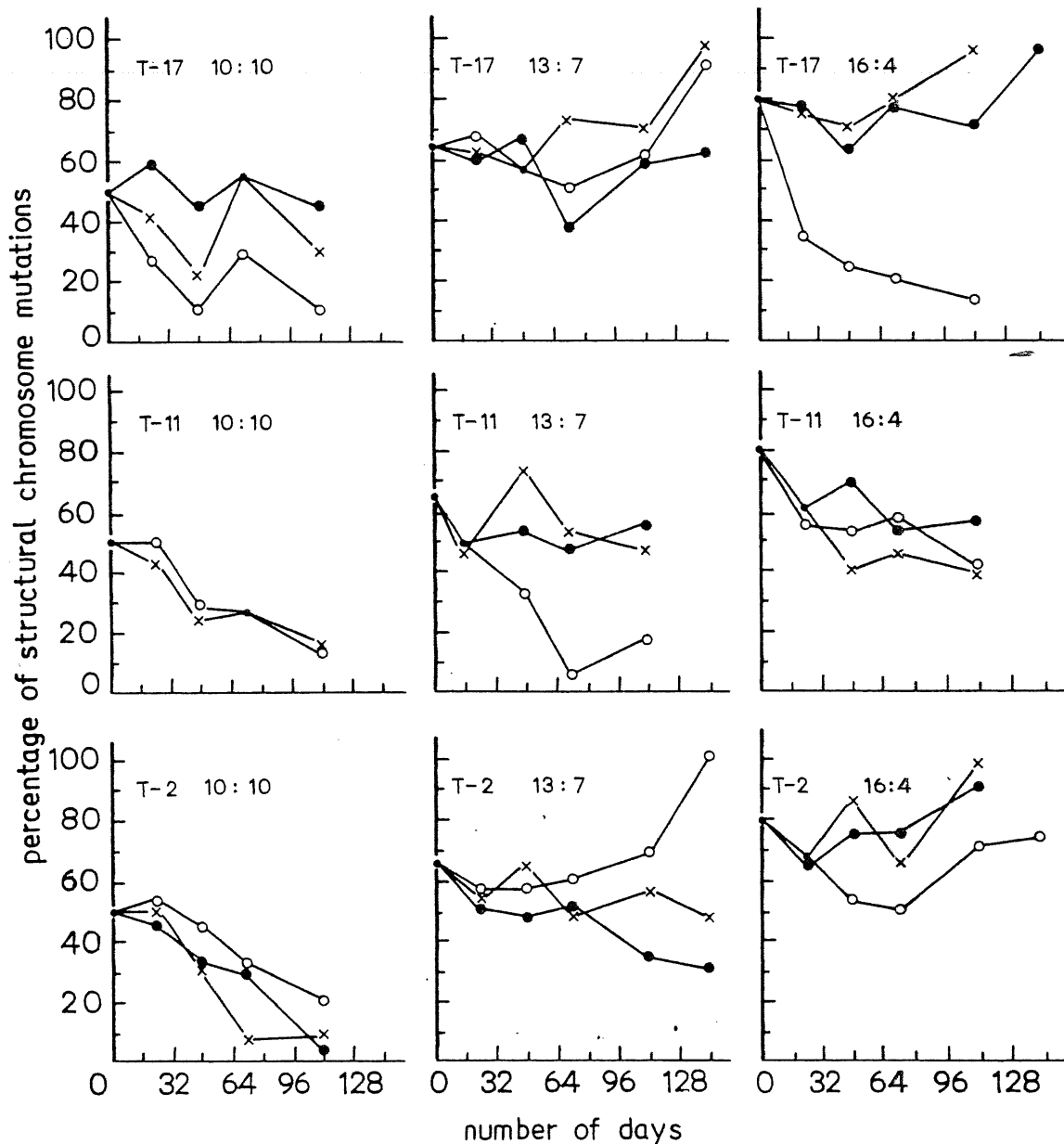


Fig. 2. The process of population displacement in experimental populations of *Tetranychus urticae*, for three different strains, homozygous for a structural chromosome mutation (T): strain T-17, T-11 and T-2. The experimental populations were set up by mixing T/T females and +/+ females (wild-type) in ratios of 10:10, 13:7 and 16:4 respectively. Each experiment was triplicated (O—O; X—X and ●—●). The percentage of the T karyotype is given (vertically) in relation to the time (horizontally).

with the curves of Figure 2. Figure 3 shows that at the 16:4 release ratio of T-2 females to +/+ females, the three replicates with supplementary male releases showed an increase in the frequency of the T karyotype up to complete displacement of the wild-type karyotype. In the corresponding experi-

ments without male releases, only in two of the three populations an increase was observed in the percentage of the T karyotype.

At the 13:7 release ratio of T/T females to +/+ females respectively, two populations showed complete displacement of the + karyotype, while



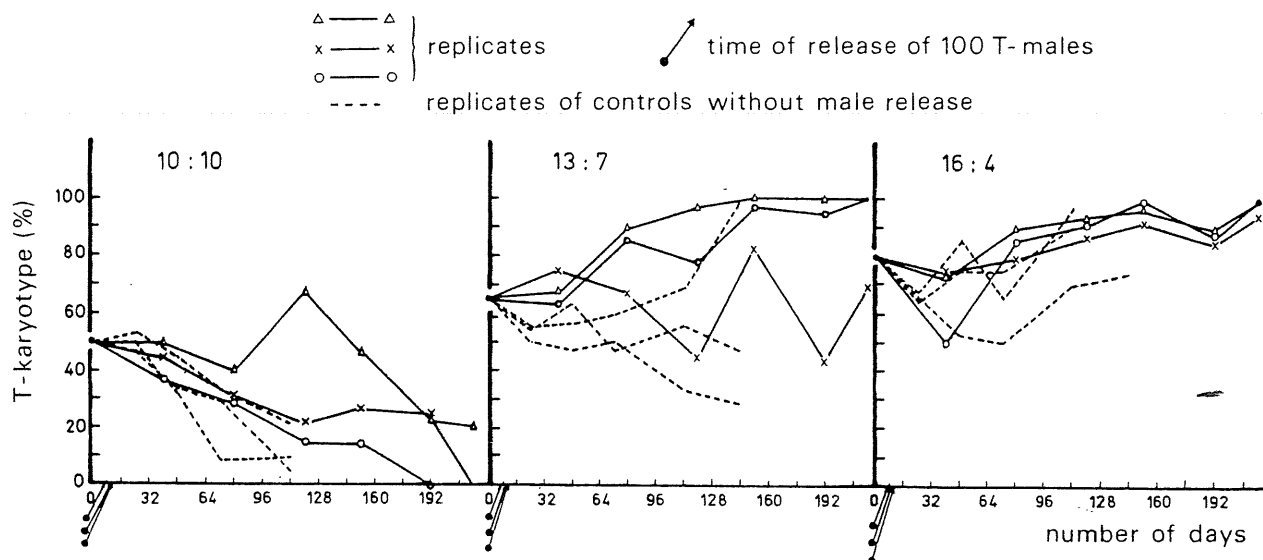


Fig. 3. The effect of triple release into the experimental population of 100 T-2 males, on each of the days 7, 9 and 11 respectively, on the process of displacement of the standard karyotype by the T-2 karyotype. The experiments on strain T-2, presented in Figure 2, without additional T-2 male releases, served as controls (presented as broken lines). Cf. Fig. 2.

in one population an equilibrium was established between the T and + karyotypes. This may be compared with one complete displacement and two declines by the T-2 in the absence of male releases at the 13:7 ratio.

In the experiments initiated with 10 T/T and 10 +/+ females, followed by three releases of 100 males, the T karyotype was exterminated after about 200 days but the rate of extermination was slower than in the experiments without male releases.

#### Computer simulation of population displacement

##### Comparison of the models

Since the mating probability ( $p_n^T$ ) is decisive for displacement, the relative contribution of a generation to the male population of its successive generation will determine the effect of overlapping generations versus discrete generations on the rate of karyotype displacement. During the process of displacement of the wild-type karyotype by the T karyotype, the mating probability  $p_n^T$  of generation  $n$  is always lower than in generation  $n + 1$ . Therefore, the existence of overlapping generations is expected to have a retarding effect on the displacement process compared to situations with discrete generations.

For a highly fertile arthropod, like *Tetranychus urticae*, this contribution of males of generation  $n$  to insemination of females of  $n + 1$  will be small under circumstances of unrestricted growth. This is demonstrated in the results of the simulations for the case of equal reproductive potential, viability and sex ratio of both strains in Figure 4 F; the differences between the two models are rather small. However, when the karyotype-specific data of the life table are supplied to both models rather large differences were found (Fig. 4 E). Apart from the minor effect of overlapping generations this deviation is caused by the age-dependent character of the production of male offspring. In the first part of their adult lives female spider mites of the standard strain produce effectively 1.8 times more males, than are produced by the T-2 females (Feldmann, 1980).

In the second part the trend is reversed, although the effect is less extreme (0.85 times more males from wild-type than from T-2). Of course the age-structured model reacts to this phenomenon with an initially higher mating probability for the + males. On the other hand the discrete-generation model reacts more directly on the weighted total reproduction ( $B = 1.12$ ) and mean sex ratio, resulting in an 'advantageous' value of  $B$  and a less 'disadvantageous' value of the sex ratio of the T

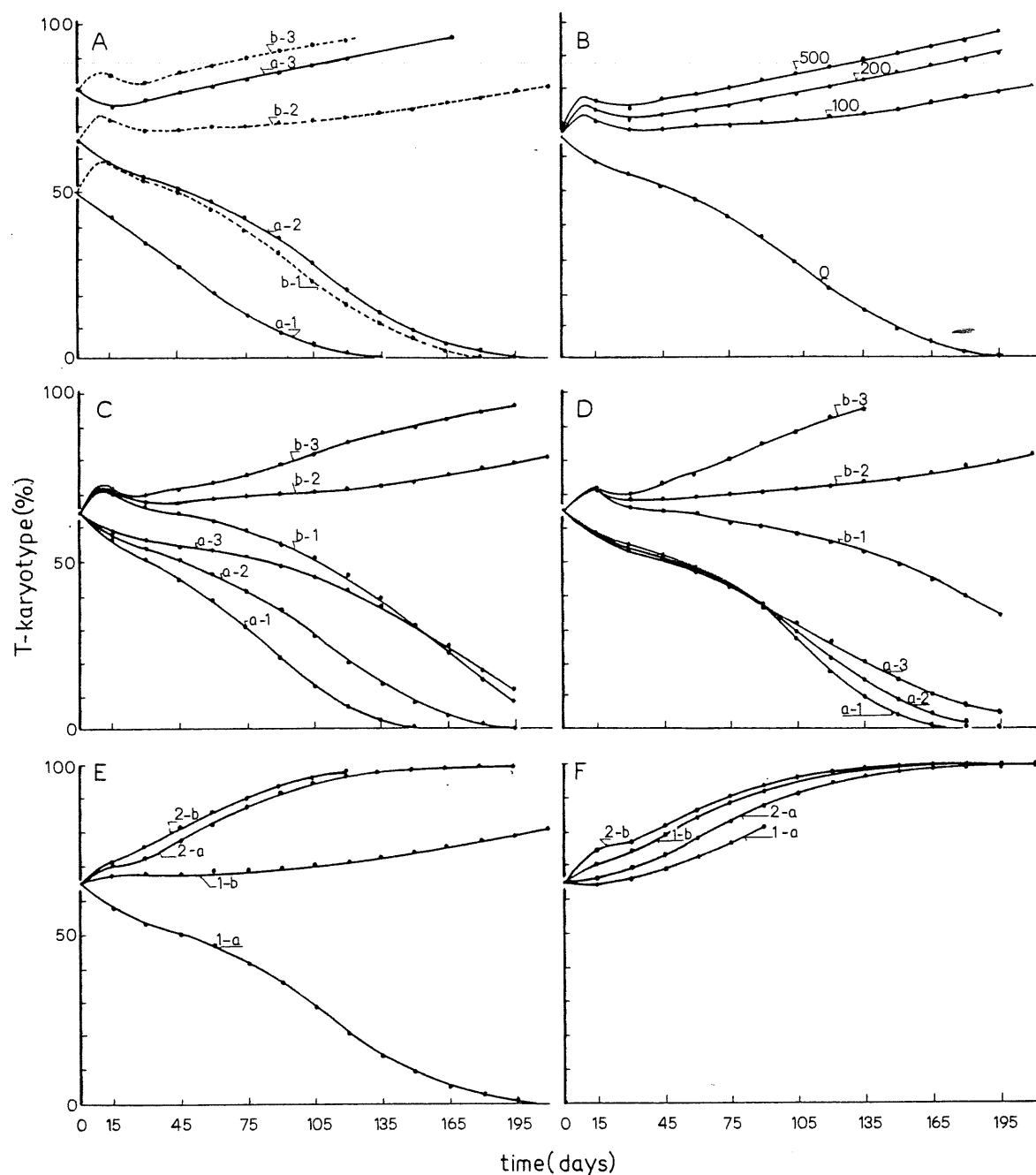


Fig. 4. The effect of varying different parameters on the output of the model simulating displacement, of a standard karyotype by a rearranged karyotype in *Tetranychus urticae* populations. The combinations of different parameters were as follows: (A) Different initial T-karyotype percentages (Graphs 1, 2 and 3 for 50, 65 and 80% respectively) either with (b) or without (a) T-male release; (B) Different numbers of T-male release (0 - 100 - 200 - 500 respectively) at an initial T-karyotype percentage of 65%; (C) Different T-male mating competitiveness values (Graphs 1, 2 and 3 correspond to values of 0.75, 0.88 and 1.00 respectively) either with (b) or without (a) T-male release; (D) Different levels of hybrid fertility (Graphs 1, 2 and 3 compared to values of 50, 35 and 20% respectively) either with (b) or without (a) T-male release; (E) Overlapping (1) and discrete (2) generations either with (b) or without (a) T-male release; (F) Overlapping (1) and discrete (2) generations either with (b) or without (a) T-male release; equal reproduction, sex ratio and other fitness parameters for both the standard and T strain.

The input data for the simulations A-E are measured data (Feldmann, 1980), except for the variable under test in each set of simulations. 100 T-males were released on each of the days 7, 9 and 11 unless otherwise stated. A-D are based on the overlapping generation model only.

population. It is therefore concluded, that the sequence of age-dependent events has to be included in the analysis of the population experiments. Therefore the age-structured model is used for all further simulations.

#### *Sensitivity analysis*

For the analysis of the sensitivity of the model for different population characteristics, those situations are of interest, which are near to the unstable equilibrium, where the displacement process is most sensitive to perturbations. Therefore, according to the results presented in Figures 2, 3 and 4, the initial ratio of 13 T/T females to 7 +/+ females was selected.

As discussed before, the parameter  $p_n^T$  is of crucial importance. The initial release of females ensures a continuous production of young and competitive T-males, during the whole reproductive life span of the parental females.

If instead of T/T females only T males were released, repeated introductions of young T males would be necessary for obtaining an effect which is comparable with the release of T/T females. The importance of T-male mating competitiveness relative to + males, for the displacement process, is presented in Figure 4 C, for some realistic values (from table 1 in Feldmann, 1980).

The alteration of the value for T-male mating competitiveness (0.75, 0.88 and 1.00) only had a clear effect on population displacement in conjunction with the threefold release of 100 T males, causing a situation near the unstable equilibrium.

Only at an initial release ratio of 16 T/T females to 4 +/+ females, does the model predict displacement of the + karyotype (Fig. 4 A) without male releases. The effect of a threefold release on days 7, 9 and 11 respectively of different numbers of T males is given in Figure 4 B at an initial ratio of 13 T/T females to 7 +/+ females. The release of T males had a significant effect on karyotype displacement, but increasing the number released at each of the three releases above 100 yielded diminishing returns.

The effect of variation in development time and a slightly shorter developmental time of the males was shown by the simulations to be of minor importance within the range of realistic values. However, the mean developmental time from egg to adult female is extremely important (see Fig. 6). A

developmental time of the T/T homozygotes shortened by more than half a day allows displacement of the wild-type population at the 13 T/T : 7 +/+ ratio without release of males. The importance of small changes in development time was already pointed out by Lewontin (1965). He concluded that for highly reproducing species small absolute changes in developmental rates of the order of 10% are roughly equivalent to increases in fertility of about 100%.

Figure 4 D gives an indication of the effect of varying the level of hybrid fertility. The rate of the displacement process is positively correlated with the degree of inter-strain sterility. The ideal genetic system for displacement would be one causing complete sterility in crosses and full fertility in intra-strain matings.

#### **Discussion**

##### *Comparison of the experimental results with those of computer simulation*

Although the results of the population experiments are subject to stochastic processes and only three replicates were carried out, some qualitative conclusions can be drawn with regard to the validation of the simulation model (compare Fig. 3 with Fig. 4 A). The decrease of the T-2 karyotype frequency at the 10 T/T : 10 +/+ and 13 T/T : 7 +/+ initial ratios without T-male releases is in agreement with the experiments except for one replicate at the 13:7 ratio. The effect of the male releases at these ratios is also demonstrated in the model.

It should be noted that the simulated rate of displacement was always very close to at least two replicate experiments. The reduced mating competitiveness and reduced production of males of strain T-2 (Feldmann, 1980), are the main sources of disadvantage for the T-2 strain in the simulation runs. Figure 4 C shows however, that equalization of male mating competitiveness, without additional male releases, was not sufficient to achieve displacement of the + karyotype at a 13:7 initial ratio of T/T to +/+ females. Only in combination with T-male releases did an increase of T-male mating competitiveness to 1.0 result in displacement of the + karyotype. Without additional T-male release

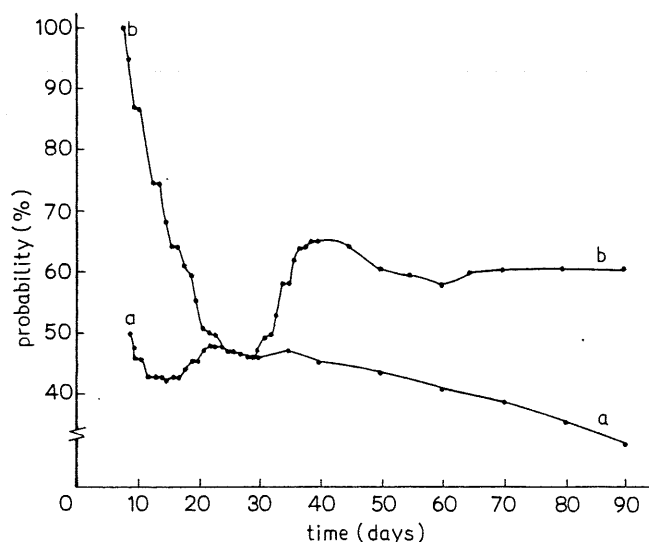


Fig. 5. Simulation studies on the effect of the release of 100 T males (on each of the days 7, 9 and 11 respectively) on the probability of females being mated by T males, during the process of karyotype displacement in an experimental population of *Tetranychus urticae*. This population was initiated from 13 T/T females and 7 +/+ females on plants on day 0. a = without T-male release; b = with T-male release.

the fixation of the T-2 karyotype was obtained by increasing the initial frequency of the T karyotype to 0.80 (Figs 2 and 4 A). The T-male release on days 7, 9 and 11 only had a significant effect on the process of population displacement, if the initial frequency of the T karyotype was close to the point of unstable equilibrium (see Figs 3 and 4 A). Figure 4 B shows that the triple release of 100 T males just created a situation which allowed for a very slow increase of the T-2 karyotype frequency. Doubling of the number of released T males influences the rate of displacement noticeably.

The probability of a female being mated by a T male is permanently increased if at least 100 males are released on each of days 7, 9 and 11 of the experiment (Fig. 5). Such a condition can also be obtained if one selects a T strain, which produces relatively more male progeny. There are indications in *Tetranychus urticae* that selection for high or low sex ratio is possible (Mitchell, 1972; Overmeer & Harrison, 1969) but more study on this subject is required.

Theoretically shortening of the developmental time of females would have a significant influence on the displacement ability of the T-2

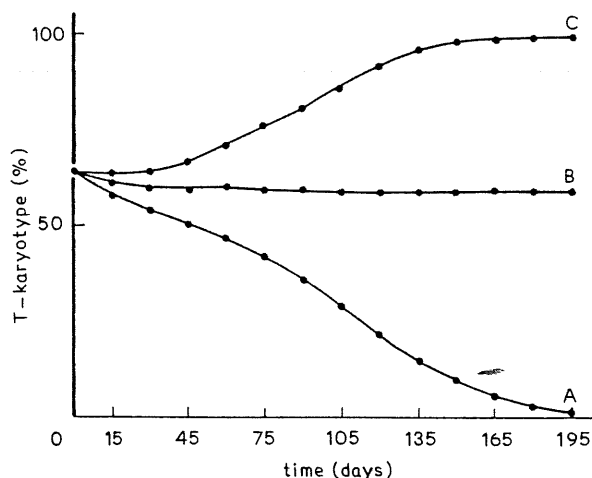


Fig. 6. Computer simulation studies on the effect of varying, within one day, the length of T/T female developmental time on the population displacement ability of the T strain. Except for developmental time, all the other parameters were kept as they were measured. The initial T-karyotype percentage was 65%, consisting of 13 T/T females and 7 +/+ females.

Graphs: (A) measured fitness differences, no T-male release; (B) as A, except for T/T female developmental time which was shortened for half a day; (C) as B, but shortening of development time by one day.

strain (Fig. 6). Without additional T-2 male releases shortening of the developmental time of T-2 females by half a day results in a situation of unstable equilibrium. Selection for faster development and increased fecundity has been shown to be the result of colonizing a wild strain of *Anastrepha suspensa* in the laboratory (Chambers, 1977). It would be worthwhile to study in *Tetranychus urticae* the possibility of selection for faster developmental rate and increased fecundity. The increase in fecundity of a T strain should, however, only lead to the production of more T males because, firstly, in that case the probability of females being mated by a T male ( $p_n^T$ ) has increased and secondly, males do not cause economic damage to the crop.

A theoretical decrease in the level of hybrid fertility of strain T-2 hybrids (T/+ females) from 35% to 20% would only have a positive effect on population displacement in conjunction with additional T-male releases (see Fig. 4 D). An increase in the level of hybrid fertility from 35% to 50% results, according to the computer simulations, in a decrease in the ability of the T strain to displace the standard karyotype (see graphs b-2 and b-1 in Fig. 4 D). T-2 hybrids with a fertility of 50 percent still

contribute such a large number of males to the pool of males, that three additional releases of 100 T males on days 7, 9 and 11 of the experiment, are of relatively minor importance.

Selection for higher levels of hybrid sterility is possible by combining more than one chromosome mutation (Overmeer & van Zon, 1973). However, in this case there is also the possibility of negative fitness effects, associated with each of the mutations and interaction between them. Structural chromosome mutations were made homozygous following X-irradiation of sperm of males of strain T-17 (Feldmann, unpublished results). Three new strains were obtained with full viability and fertility of the homozygotes and with very low hybrid fertility (about 10–15 percent). The population displacement experiments with each of these lines against the standard strain (wild-type) showed that each of these strains was unable to displace the + karyotype from the population even at an initial ratio of 16 T/T females to 4 +/+ females (without additional T-male releases).

Theoretically, complete hybrid sterility would be the ideal situation if the chromosome mutation homozygotes have full fitness because firstly, the rate of + karyotype displacement is inversely correlated with the level of hybrid fertility and secondly, in the case of practical application of the displacement technique, 'beneficial genes' which have been introduced into the T strain, must not recombine with the wild-type genome. Such genetic recombination of the 'beneficial genes' with their wild-type alleles would render the whole procedure of karyotype displacement useless. The ideal condition for karyotype displacement is partly fulfilled by compound chromosomes, known in *Drosophila melanogaster* and in a few insect species of economic or medical importance and by bidirectional cytoplasmic incompatibility, known from some mosquito species (Laven, 1967). Strains, homozygous for compound autosomes have in general an intra-strain fertility of 25 percent but have an inter-strain fertility of 0 percent (Holm, 1974). Positive results both in the field (McKenzie, 1976) and in cages (Fitz-Earle *et al.*, 1975) on population displacement experiments were carried out with *Drosophila* compound stocks. But due to the low fitness of the compound chromosome strain, the whole displacement process is rather sensitive to immigration of wild-type individuals (McKenzie, 1977).

Recombination in compound heterozygotes of genes located on non-compound chromosomes is possible at very low frequency. Thus the gene to be driven into the population (conditional lethal or vector refractoriness) would have to be linked to the compound chromosome (Fitz-Early *et al.*, 1973).

Population displacement in cage populations of *Culex fatigans* of a native karyotype by the karyotype of a strain possessing bidirectional cytoplasmic incompatibility, were also successful (Curtis & Adak, 1974; Curtis, 1976). The expectation of complete cytoplasmic incompatibility was however, not fulfilled since recombination between the native karyotypes and the released karyotype occurred at a low frequency.

The absence of complete hybrid sterility in complex chromosomal rearrangements in *Tetranychus urticae* (Overmeer & van Zon, 1973) would have the consequence that the gene which was to be driven into a pest population, must be completely linked to the driving mechanism. This could be achieved if this gene was at, or very close to the chromosomal breakage point or if cross-over suppressors such as chromosomal inversions were used.

#### *Effect of random fluctuations of initial T-karyotype frequency on population displacement*

The population size at the start of the described experiments was very small (only a total of 20 females) which allows for random changes in chromosome mutation frequency because of early death of one or more of these females, due either to chance or damage due to handling. This will have an important effect on populations with the chromosome mutation frequency near its unstable equilibrium. At this point, chance deviations of the frequency of the chromosome mutation in the population would be decisive for the direction of the process of population displacement. This process may have been responsible for the fact that in one of the three populations initiated with 16 T/T females of T-17 and 4 +/+ females (see Fig. 2), the chromosome mutation was exterminated, in contrast with the fixation of T-17 in the other replicates. A corresponding observation was made in the experiments involving T-11 at the 13:7 ratio of T-females to standard females (see Fig. 2). The T karyotype in one population went rapidly to exter-

mination while in the other two replicates an oscillation around 50% occurred. The experiments with strain T-2 give an example of a possible effect of chance deviations of the T-karyotype around its point of unstable equilibrium (Fig. 2; T-2, 13:7). In one population, the T karyotype went to extermination; in the second population the T karyotype went with increasing velocity to fixation after passing the point of unstable equilibrium; in the third population, the T-2 karyotype oscillated around 50 percent throughout the experiment (144 days). The graphs in Figures 2 and 3 show a coherent pattern, with extermination or fixation of the T karyotype if the percentage of the T karyotypes after the first few generations was obviously below or above the point of unstable equilibrium respectively. In these situations small changes in the T karyotype frequency either due to random fluctuations or to release of T males (Fig. 3) will not result in a significant effect on the direction of selection.

#### *Comparison of the results with data from the literature*

The results of the population displacement experiments show that two out of three different structural chromosome mutations could successfully eliminate the wild-type karyotype from a population without additional male releases. Up to now 20 different T lines, homozygous for a structural chromosome mutation, have been tested for their population displacement abilities (unpublished results). Of these 20 T lines only 4, including T-2 and T-17 which have been described here showed population displacement if the T karyotype was initially at a level of 65 percent (13 T/T females to 7 +/+ females) or higher. These results are favourable in comparison with similar experiments in other species. Trials of population displacement using a *Drosophila* translocation in a cage population failed, even at an initial ratio of 9:1 of translocation homozygote flies to standards. Field experiments on population displacement with a translocation homozygote in *Aedes aegypti* were not successful (Lorimer *et al.*, 1976). Both trials most probably failed because of the reduced fitness of the translocation homozygotes used. Both the translocation strain of *Drosophila* and of *Aedes aegypti* were homozygous for a recessive genetic

marker, facilitating monitoring of the translocation in the population. Selective disadvantage of a genetic marker, linked with a translocation has been proved to contribute to the failure of some population displacement experiments. The wild-type eye-color marker instead of red eye (*re*) in the homozygous translocation strain of *Aedes aegypti* improved considerably both the recapture and the effectiveness of ovitraps following field releases of translocation-bearing mosquitoes (Lorimer *et al.*, 1976). Comparable information in the marker *ruby eye* was obtained in *Culex fatigans* (Curtis & Adak, 1974).

In *Drosophila* the population-displacement ability of a compound strain was considerably improved after replacing the marker *ri* by its wild-type allele. Selective disadvantages in *Drosophila* were found for the markers *bw*, *b*, and *dp* (Childress, 1972, Fitz-Earle *et al.*, 1973, 1975).

Corresponding information was obtained in *Tetranychus urticae* experiments. Each of six *Tetranychus urticae* strains, homozygous for a structural chromosome mutation and the recessive eye-color marker *we* (white eye), were eliminated from an experimental population following competition with the standard strain (wild-type), initiated with a ratio of 16 T/T females to 4 standard females. Another indication of the selective disadvantage of a genetic marker in *Tetranychus urticae* was observed following the competition in laboratory populations of each of seven different T lines in an initial ratio of 10 T/T females to 10 standard females, homozygous for the recessive eye- and body-color marker *alb* (albino). Four of these seven T strains (including the strains T-2, T-17 and T-11) increased up to 90 percent within 45 days after the initiation of the experiments. This is in contrast with the results shown in Figure 2 because firstly the karyotype of T-11 was eliminated from a population following competition against standards, possessing the wild-type allele of *alb*, even at an initial T-11 frequency of 0.85. Secondly, T-2 and T-17 both had points of unstable equilibrium at about 65% when competing against wild-type standards. Thus the relative fitness of the T-strains compared with *alb* standards is higher than when they were compared with wild-type standards, indicating the negative contribution of the genetic marker to fitness. A reduction in viability is often found in association with chromosomal translocations.

These effects are mostly recessive and are supposed to be position effects or gene damage at or close to the chromosomal breakage point (Catcheside, 1945; Sobels, 1972). Strain T-2 of *Tetranychus urticae*, which was tested for various components of fitness (Feldmann, 1980) showed a small reduction on male mating competitiveness but a slight increase of the net reproductive rate in comparison with wild-type. The reduction in fitness of strain T-2 was reflected in the level of the point of unstable equilibrium being at about 0.65 (see Fig. 2) instead of 0.50 in the case of equal fitness of the T-strain and the standard strain (Curtis, 1968b; Curtis & Hill, 1971).

*Some considerations concerning practical application of the Population-Displacement Technique in Tetranychus urticae*

The immigration of wild-type material in greenhouses is poorly studied. But if in the greenhouse a resident population, having the desired genetic traits, can be maintained below the economic threshold, then this resident population will be resilient towards immigration (Curtis, 1968b; Dietz, 1976; McKenzie, 1977; von Ende, 1978). A study is needed on the balance of selective values exercised on a genotype which occurs at a low frequency (e.g. immigrants) and is subject to extermination on outbreeding with the resident greenhouse population, but which is resistant to control measurements (either high or low temperature or acaricides). The outcome of studies on the importance of immigration and the balance of prevailing selective values will be very important for the assessment of the value of the control technique (Comins, 1977). However, immigration is for greenhouses basically a technical problem which may be limited by applying adequate sanitary precautions.

The capacity of a resident greenhouse population, having the desired genetic traits, to counteract the effect of immigration, depends on the exchange of genes between separate colonies in the greenhouse. Thus it is important that the density of the resident population, having the desired genetic traits, is as high as is economically acceptable. The diffusion of males and females within a greenhouse area and the interaction between different genotypes are of fundamental importance to the proposed technique. The present computer model can

be extended to include the effect of dispersal. To this purpose, this model simulates population growth in units, representing leaves, plants or infestation foci, which are coupled via dispersal rates.

The results demonstrate that population displacement in *Tetranychus urticae* has been successful but the following problems are significant when practical application is considered:

(a) Recombination of resistance genes (either temperature- or acaricide-resistance genes) from the wild-type population with the sensitive population possessing the chromosomal rearrangement;

(b) Immigration of wild-type individuals;

(c) Patchy (discontinuous) distribution of the species within a greenhouse;

(d) Lack of information on the economic threshold in relation to population density;

(e) Estimation of the minimum level of population density at which: (1) immigration can be effectively 'buffered' by the resident population having the desired genetic traits; (2) the process of population displacement can take place; (3) interchange between colonies is possible.

These problems are not independent and are also not exclusively associated with genetic control methods. For example (b), (c) and (d) are problems which are also important for both chemical and biological control (predators). Topic (c) is of major importance for biological control in which predators have to trace isolated colonies of *Tetranychus urticae*. Each of the problems, mentioned above (a to e) can seriously limit the applicability of the proposed genetic control method. Topic (a) is perhaps the major obstacle for the application of the population-displacement technique. However, the introduction and isolation of chromosomal rearrangements in *Tetranychus urticae* is very easy. Thus it is possible to screen a large number of lines for lack of recombination with acaricide-resistance genes. More promising are conditional lethals in this respect. Conditional lethals, such as temperature-sensitive lethals, can be induced at a large number of different loci. Thus by treating a line, homozygous for a chromosomal rearrangement, with a mutagen (Ethylmethanesulphonate) or conversely, irradiating a line, homozygous for a temperature-sensitive mutation, translocations and temperature sensitives in a variety of relative positions could be produced. It is very likely that some

of these lethals would be located so close to the breakage point that recombination with its wild-type allele would be absent as in the housefly (McDonald & Overland, 1973) and in *Drosophila* (Kaufman & Suzuki, 1974).

The population-displacement system using bidirectional cytoplasmic incompatibility is more powerful than a system based on compound chromosomes due to the large reduction in fitness of the released individuals carrying the compound chromosomes. Compound-chromosome mutation strains have in general an intra-strain fertility of only 25 percent (Holm, 1974); strains exhibiting bidirectional cytoplasmic incompatibility following outcrossing, may have as homozygous translocation strains, normal intra-strain fertility. The cytoplasmic incompatibility system is also more powerful than a system based on a chromosomal translocation because (a) homozygous translocation strains exhibit partial hybrid fertility following outcrossing and (b) the reduction in fertility is in the  $F_1$ -hybrids instead of the absence of hybrids in the case of cytoplasmic incompatibility. In relation to the experiments on *Tetranychus urticae* published here, a third factor favouring the cytoplasmic incompatibility system for population displacement deserved attention: (c) Only the hybrid female of *T. urticae* exhibits reduction in fertility; the hemizygous male, having either the + karyotype or the T karyotype, is fully fertile.

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## Appendix

*Structure of the simulation model for the case of overlapping generations.* The model is available from the authors on request. It is written in CSMP-computer language and can be divided in three segments:

(1) 3 submodels (MACRO), simulating development of eggs, juveniles and preoviposition females (BOXCAR), female ageing and reproduction (REPROD), plus male ageing and age dependent competitiveness (MALE).

(2) INITIAL segment, containing input data and initial calculations.

(3) DYNAMIC segment, in which the growth and displace-

ment process is formulated in successive sections (= groupings of statements with a heading).

In the beginning of the program two parallel arrays of rate variables (RY (INDEX)) and state variables (Y (INDEX)) are declared. The state variables express the numbers of individuals and the rate variables express the rate of change in these numbers, as a resultant of the inflow from the preceding class (Y (INDEX-1)) and the outflow to the next class (Y (INDEX+1)). These rate variables are computed consecutively ( $1 < \text{INDEX} < 200$ ) in the submodels at each time interval.

The submodels are invoked in the dynamic segment of the model in a distinct order (starting from eggs and ending with adult). These so called MACRO-calls are accomplished as follows:

$$\begin{array}{ccccc} \text{number} & \text{outflow} & & & \text{developmental} \\ \text{of} & \text{to the} & = \text{BOXCAR} & & \text{rate} \\ \text{mites} & \text{next stage} & & & \\ & & \text{dispersion} & \text{inflow from} & \text{number of} \\ & & \text{in} & \text{the preced-} & \text{intra-} \\ & & \text{development} & \text{ing stage} & \text{stage} \\ & & & & \text{classes} \end{array}$$

REPROD and MALE are virtually the same type of boxcar-models, except for another arrangement of inputs and outputs. REPROD computes, additional to the number of females, the reproduction and sex ratio of the offspring. MALE computes additionally the weighted age dependent competitiveness (intra-karyotype).

After computation of the rate variables, the state variables are calculated by rectilinear integration over a sufficiently short time interval (DELTA):  $Y(\text{INDEX}) = Y(\text{INDEX}) + RY(\text{INDEX}) \times \text{DELTA}$ .

Except for the simulation of the age dependent phenomena and dispersion in developmental time, the structure of this model is equivalent to that of the model for discrete generations.

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