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The Effect of Immigration on Genetic Control

A Laboratory Study with Wild and Compound Chromosome Stocks of *Drosophila melanogaster*

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Summary. Immigration by wild type flies into an established compound chromosome control zone was studied in the laboratory using discrete generation population cages. Immigration rates of less than 10 % per generation by virgin migrants were unlikely to disrupt the zone. However, the zone could be disrupted by immigration rates of 0.5 % if the migrants had mated. The curvilinear relationship between the number of generations to fixation of the migrant genotype and the immigration rate suggested a possible equilibrium between immigration rate and the maintenance of a control zone. - The importance of the results to the strategy of a particular control program is emphasized, as is the need for an integrated multi-disciplinary approach to insect pest management.

Introduction

Control of insect populations by the use of genetics has been suggested by many authors (see Davidson 1974; Pal and Whitten 1974; Whitten and Foster 1975 for recent reviews). While genetic control offers the potential for manipulation not available with more conventional methods of control, many unique operational difficulties may exist (Pal and LaChance 1974). The population biology of the target species may also need to be more rigidly defined than in the case of conventional methods (Foster *et al.* 1975). Indeed, the level of understanding of the population biology and the prospect of a successful genetic control program would seem to be directly related (Whitten and Foster 1975; McKenzie 1976).

One proposed method of genetic control is the use of compound chromosome individuals to replace a field population (Foster *et al.* 1972). Trials with *Drosophila melanogaster* in laboratory (Childress 1972; Fitz-Earle *et al.* 1973; Fitz-Earle 1975) and field cages (Fitz-Earle *et al.* 1975) have yielded successful results. The results of releases into native populations of this species have been less clear cut (Cantelo and Childress 1974; McKenzie 1976) although McKenzie's (1976) results gave some cause for guarded optimism about the general potential of the technique. In that study, the compound stock released into the cellar population of the "Chateau Tahbilk" vineyard, in Victoria, successfully bred and replaced the field population for a considerable period. The subsequent replacement of the compound stock by wild flies was explained by the immigration of inseminated wild-type females into the cellar.

The effect of immigration into a target zone is a potential problem in any control program. It is relevant to know what immigration rate can be tolerated in an established control zone (Proverbs 1974). Theoretical considerations indicate that the critical rate is a function of the relative fertilities of the immigrants and the stock used for genetic control (Dietz 1976). The intrinsic deficiency of egg hatch of a compound chromosome *D. melanogaster* stock (Foster *et al.* 1972) ensures such stocks have a reduced fertility relative to wild type. Therefore, given the results of the vineyard study, it is of interest to consider the effect of immigration into a compound population under defined laboratory conditions. Immigration by either virgin or inseminated individuals has been considered as while *D. melanogaster* immigrants are likely to have mated (McKenzie 1976), this may not be true of other species. Such a difference in the type of migrant may impose important limitations on a particular control program.

Methods and Materials

Experimental procedures and stock maintenance were carried out at 25°C in constant light.

The immigrant wild type stock (designated ±) was derived from the progeny of single inseminated *D. melanogaster* females collected outside the cellar of the "Chateau Tahbilk" vineyard (McKenzie 1974) and maintained in mass culture on standard medium.

The compound stock was derived by irradiating (approximately 3400 rads of Co-60 γ radiation at 220 rads per min) virgin ± females when 2-4 days old and

then mating to C(3L)P3, ri; C(3R)P3, sr males (obtained from Pasadena). C(3L)P3, ri; C(3R), ± progeny were maintained and males crossed to irradiated ± females (procedure as above). C(3L)P3, ri; C(3R), ± progeny were mass cultured and this stock (designated C ri) was used in the experiments. A full description of the markers is provided by Lindsley and Grell (1967).

Comparison of the Stocks

Any comparison of the stocks is only relevant to the particular population cage design used. A discrete generation design, with a restricted oviposition period, minimizes the variables associated with a more complex overlapping generation design and has been chosen for this reason.

Mating behaviour

Fifteen 2-3 day old virgin flies of each sex of both stocks were placed in a mating chamber (McKenzie 1976). Copulating pairs were removed and each mating partner was scored for the marker ri. Results were recorded for 16 trials, each of 60 minutes duration.

Fecundity and fertility

The procedures detailed were carried out separately for each stock. Five 2-3 day old virgin males and females of a stock were placed in an empty 280 ml culture bottle. The bottle opening was then covered by a watchglass containing a thin layer of standard medium. After 24 hours the flies were discarded and the number of eggs oviposited was recorded for each of 10 replicates.

Ten replicates, each of 25 eggs, were collected and transferred to vials each containing 10 ml of standard medium. The numbers of adults emerging after development through larval and pupal stages were recorded for each replicate.

Population Cage Studies

The cages were 280 ml bottles each containing 70 ml of standard medium. Immigration was for both sexes with immigrant females being either virgin or inseminated. Unless otherwise specified, 3 replicates were carried out for each comparison.

Series a (10 %, 8 %, 6 %, 4 %, 2 %, 1 % immigration per generation)

Cages were initiated with 50 C ri males and 50 inseminated C ri females. The appropriate numbers of ± males and females were added to generate the designated immigration rate. For 1 % migration, 1 male and 1 female were added as immigrants every second generation beginning at the start of generation 2.

Flies were left in the bottles for 24 hours and then discarded. Seven days later 1 ml of yeast solution was added to each bottle. Fourteen days after the flies had been added to the bottles, by which time all progeny had emerged, progeny were removed and scored for the ri phenotype. Fifty flies of each sex were than randomly chosen from the progeny and, with the appropriate number of ± immigrants, added to a new cage to start the next generation.

The procedure was repeated until fixation of the ± phenotype occurred in all of the inseminated immigrant cages of each migration rate.

Series b (2 %, 1 % immigration per generation)

The procedure was as for series a but cages were initiated with 100 C ri males and 100 inseminated C ri females and the appropriate number of ± immigrants. Subsequent generations were established with 100 randomly chosen flies of each sex plus immigrants of both sexes. Virgin immigration was only carried out at the 1 % rate.

Series c (2 % and 0.5 % immigration per generation)

The procedure was as for series a but cages were initiated with 200 mated C ri flies of each sex and subsequent generations were established with 200 randomly chosen males and females. The appropriate number of immigrants was added each generation. Virgin immigration was carried out only at the 0,5 % rate.

Table 1. Numbers mating in 60 minutes in mating chambers containing 15 flies of each sex of the C ri and ± stocks (based on 16 trials)

Female	Male		
	C ri	±	Total
C ri	57	46	103
±	42	49	91
Total	99	95	194

χ^2_1 for 1 : 1 ratios on marginal totals, which assess the relative mating propensities of the stocks

♂ 0.08 ♀ 0.74

Table 2. Number of eggs laid in 24 hours by 5 females of the C ri and ± stocks

Replicate Number	C ri	±
1	153	243
2	298	169
3	391	266
4	315	141
5	204	266
6	182	258
7	321	361
8	251	220
9	351	265
10	120	236
Mean	258.6	242.5
s.d.	90.7	59.8

Table 3. Numbers of adults emerging after development from samples of 25 eggs of C ri and ± stocks

Replicate Number	C ri	±
1	4	18
2	3	22
3	4	21
4	4	25
5	6	19
6	5	20
7	3	17
8	8	20
9	3	21
10	4	19
Mean	4.4	20.2
s.d.	1.6	2.3

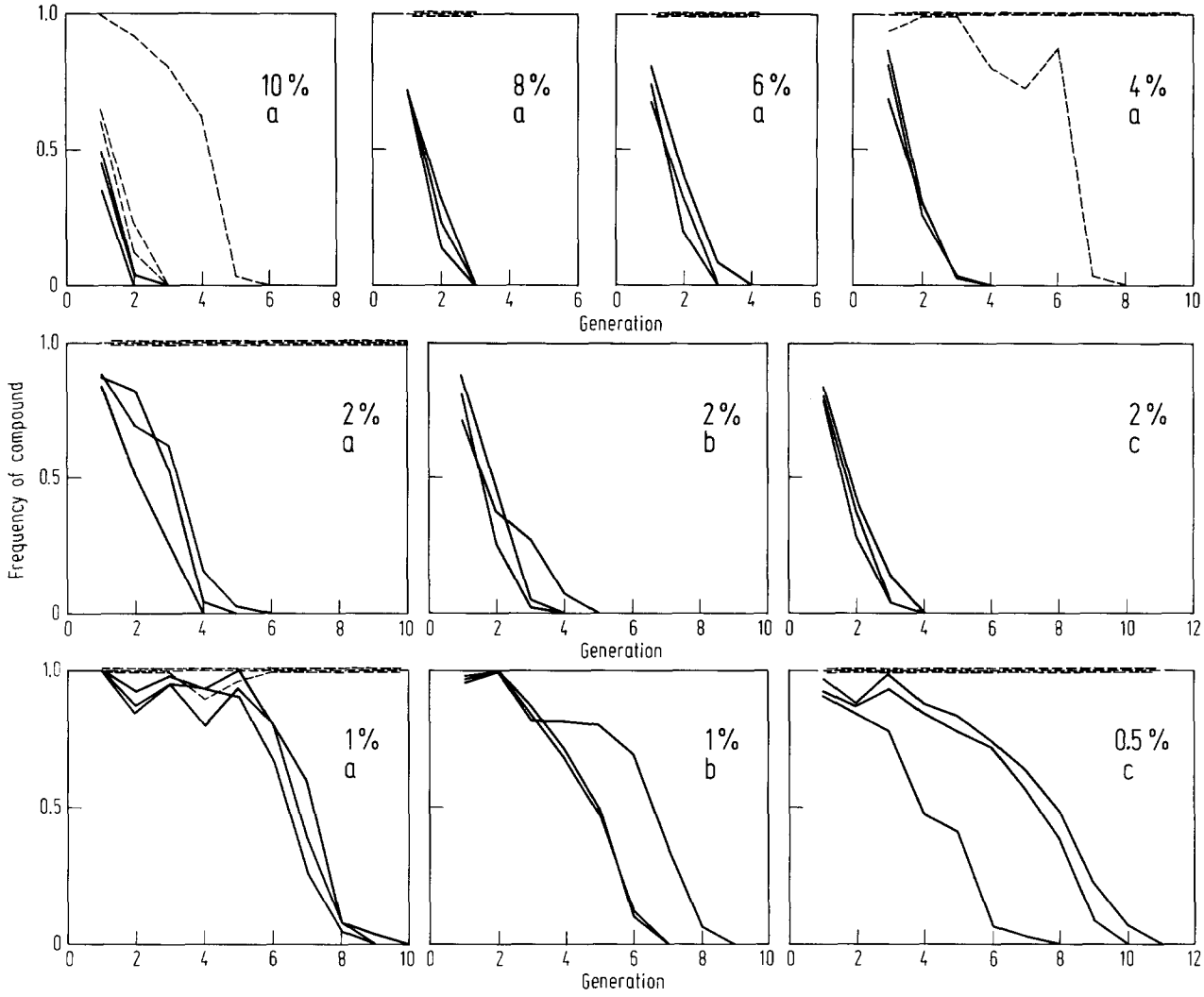

Fig. 1. Frequency of compound (C ri) individuals in population cages into which immigration by virgin (broken line) or mated (solid line) ± individuals occurs at the rates specified each generation. The number of flies of each sex, excluding migrants, used to start each generation is indicated (a = 50, b = 100, c = 200)

Results

Comparison of the mating behaviour, fecundity and fertility of the C ri and ± stocks shows only fertility is significantly different.

The mating propensities of males and females of both stocks are very similar (Table 1). More importantly, there is no indication of isolation between the stocks as the Joint Isolation Index is 0.09 ± 0.07, a value of 0 indicating random mating (see Malogolowkin-Cohen *et al.* 1965). In the fecundity estimate experiment almost all females were inseminated after the 24 hour mating and oviposition period and the number of eggs laid by either stock (Table 2) did not differ significantly ($t_{18} = 0.46$, $0.7 > P > 0.6$). The fertility of the C ri stock was estimated from Table 3 to be 21.8 % that of the ± stock. The difference is expected because of the egg hatch deficiency of a compound stock (Foster *et al.* 1972) and is highly significant ($t_{18} = 18.17$, $P \ll 0.001$).

The above results suggest that the C ri stock should be able to replace a ± population if released at a frequency above the unstable equilibrium point (Foster *et al.* 1972). For a population cage design similar to that to be used in the immigration studies the unstable equilibrium point was found to be approximately at a C ri frequency of 0.9. Therefore, for the particular universe of this laboratory population compound chromosomes can be seen as an effective transport mecha-

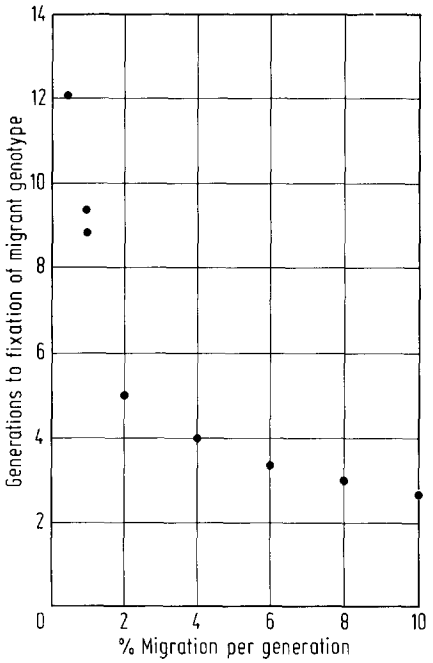

Fig.2. Standardized mean number of generations to fixation of the migrant genotype graphed against migration rate per generation by mated immigrants

nism for genetic control leading to fixation of the introduced genotype.

The effect of immigration into such an established compound chromosome area can be seen in Fig.1. All immigration rates involving inseminated females ultimately result in disruption of the compound zone. The rate of disruption depends on the rate of immigration, 10 % immigration per generation cages fixing most rapidly while fixation at lower immigration rates takes progressively longer. The density of flies in the cages may influence the results. A more rapid average fixation rate of the immigrant genotype is observed for 2 % immigration values as the number of individuals used to start each generation increases (Fig.1, 2 % a, b and c).

To allow for this effect the relationship between the immigration rate and the mean number of generations to fixation of the ± genotype for all cages has been standardized relative to the series a 2 % migration rate. The relationship is curvilinear asymptotically approaching both axes (Fig.2). For lower levels of migration this suggests the possibility of an equilibrium relationship between the rate of immigration and the maintenance of the compound zone (Dietz 1976), particularly if the possible stochastic effects in the population cages are less relevant in natural populations.

The distinction between immigration by virgin or mated individuals is obvious (Fig.1). Disruption of an established control zone appears unlikely at virgin immigration rates of less than 10 % per generation. Even when fixation of the ± genotype occurs it generally takes considerably longer than in the case of mated immigrants.

Discussion

The success of any genetic control program will depend on a careful choice of the stock to be released (Fitz-Earle *et al.* 1973; Cantelo and Childress 1974; Whitten and Foster 1975; McKenzie 1976). One replacement has been achieved the effect of immigration into the target area becomes of paramount importance. Previous studies have implicated immigration as a critical factor in the maintenance of a control zone (McKenzie 1976). The present results demonstrate that the zone may be disrupted by restricted rates of movement into an area.

Significantly, this will depend on whether immigration is by virgin or inseminated females. In natural populations most immigrant *D.melanogaster* females are likely to be inseminated (McKenzie 1976). Migrants into the cellar at "Chateau Tahbilk" are therefore capable of making a rapid contribution to the population (McKenzie 1975) and it is not surprising that the established control zone was rapidly disrupted. This is particularly true in a species with overlapping generations as longevity differences between immigrant and compound chromosome individuals may result in a more rapid displacement. Preliminary laboratory results involving the C ri and ± stocks suggest this is so as the latter stock has a significantly greater reproductive life span (for further discussion see Fitz-Earle *et al.* 1973).

The results emphasize the need for detailed information of the population biology of the species it is hoped to control. Obviously, it is important to know whether immigrants are more likely to be virgin or inseminated as this will alter the probability of establishment or maintenance of a control zone. It has been recognised that unless the zone is ecologically or geographically isolated it may be necessary to establish a barrier zone to minimize immigration from adjoining areas (Pal and La Chance 1974). The present re-

sults indicate that for such a zone to be effective the actual width will not only depend on the vagility of potential immigrants but also on their reproductive condition.

The need for an integrated approach to the genetic control of insect pests has been stressed recently (Whitten and Foster 1975; Foster *et al.* 1975). This enables the most appropriate program to be devised. Theoretical studies can be of benefit in providing a framework for the program as comparison of the present results with Dietz's (1976) model shows. If the compound zone is to persist this model predicts a critical immigration rate of the order of 1 % for the particular strains used. As male immigration was allowed in the population cages and egg samples from females indicated some multiple insemination the comparison cannot be absolute as these components were excluded from the model. However, the two approaches are in general agreement as the population cage data show that the rate of immigration by inseminated females only has to be restricted for disruption of the control zone to occur, but indicate the possibility of an equilibrium.

Pest control will be most successful when the most exploitable stage of the life cycle is defined. The definition of that stage and the flexibility available to exploit it can only benefit by a combination of ecological, genetical and theoretical studies being directed to an understanding of the population biology of the pest. In this context arbitrary divisions of methodology or discipline would seem irrelevant.

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