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Genetic control of *Lucilia cuprina*: analysis of field trial data using simulation techniques

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Summary. An analytical version of the genetic control simulation program GENCON has been used to further analyze the data obtained during field trials of genetic control of the sheep blowfly, *Lucilia cuprina*, in 1976–79. In the simulations, population trends from a nonrelease area were used as an estimate of the rates of increase that would have occurred in the target population if there had been no releases. Genetic data from the target area (frequencies of matings by released males) were used to predict the frequencies of descendants of released males, the resulting genetic death, and the effects of this on population trends. In simulations that assumed no migration and full survival and competitiveness of all field-reared descendants of released males (translocation-bearing males and males and females heterozygous for deleterious mutations), neither the predicted genetic changes nor the predicted population trends agreed well with the observed data. Further simulations suggested that reduced survival or competitiveness of field-reared descendants did not account for this disagreement, but that immigration of wild flies into the test areas was probably a major contributor to the failure to achieve suppression. However, immigration alone was not sufficient to explain all the differences between observed and expected results. Other plausible contributors to this failure were: (1) lower survival of translocation males due to the effects of a dieldrin resistance allele carried on the translocation, and (2) increased survival of immature stages of *L. cuprina* at low population densities.

Key words: Genetic control – Simulation – Migration – Translocation – *Lucilia cuprina*

Introduction

Field trials of genetic methods for controlling the sheep blowfly, *Lucilia cuprina*, conducted near Canberra in 1976–79, demonstrated the ability of sex-linked translocation males released as mature larvae to complete development and mate with native females. However, suppression of the target populations was not demonstrated. This failure was attributed to several causes, including inadequacy of the release method under the prevailing ecological conditions, inadequate rearing capacity, insufficient prerelease ecological data, and genetic instability in the mass-rearing colony. In addition, the possibility that migration of native flies into the test populations influenced the outcome of the trials was recognized, but the data did not permit definite conclusions (Foster et al. 1985; Vogt et al. 1985).

The genetic structure and properties of the field-female killing systems tested in 1976–79 have been described elsewhere (Foster et al. 1985, 1988), but are reviewed briefly here. Released males carried a Y-autosome translocation involving two autosomes, each carrying the wild-type allele of a recessive eye pigment mutation. Homozygotes for the mutations are white or yellow-eyed, and fail to survive to reproductive maturity under field conditions (i.e., the mutations are effectively recessive lethals in the field) (Whitten et al. 1977; Whitten 1979). Because the translocation carries both the male determiner (on the Y) and the wild-type alleles of the eye mutations, released males have normally pigmented eyes. Since male recombination in *L. cuprina* is rare (Foster et al. 1980), the wild-type alleles are inherited through males. The mutant alleles are initially transmitted to female offspring, then in subsequent generations to both sexes. Genetic death in the target population arises both from the semisterility associated with the translocation,

Table 1. Generation times, estimates of population densities, rates of increase, and effective release rates during Wee Jasper and Boorowa trials

Generation No.	Trapping period[a]	Mean standardized female catch		Rate of increase (non-release)	Proportion of matings by released males
		Release	Non-release		
Wee Jasper					
1	13/10/76– 4/11/76	3.96	9.90		0.038
2	8/11/76–30/11/76	5.07	32.15	3.25	0.200
3	6/12/76–27/12/76	32.18	63.69	1.98	0.131
4	4/ 1/77–17/ 1/77	12.60	19.89	0.31	0.032
5	24/ 1/77– 7/ 2/77	8.28	15.63	0.79	0.071
6	14/ 2/77– 1/ 3/77	6.62	6.61	0.42	0.032
7	7/ 3/77–21/ 3/77	5.25	16.57	2.51	0.204
8	29/ 3/77–12/ 5/77	6.01	41.79	2.52	0.252
9	28/ 9/77– 3/11/77	0.61	2.83	0.07	0.603
10	9/11/77–28/11/77	3.76	30.25	10.69	0.467
11	5/12/77–19/12/77	12.01	39.90	1.32	0.235
12	3/ 1/78–16/ 1/78	1.97	0.63	0.02	0.221
13	25/ 1/78– 7/ 2/78	1.27	4.65	7.38	0.129
14	13/ 2/78–27/ 2/78	4.46	11.00	2.37	0.282
15	6/ 3/78–22/ 3/78	9.66	15.60	1.42	0.160
16	28/ 3/78–22/ 5/78	18.00	28.82	1.85	0.164
Boorowa					
1	17/10/78–30/10/78	1.30	2.42		0.548
2	6/11/78–28/11/78	15.58	28.04	11.59	0.554
3	6/12/78–19/12/78	39.41	75.39	2.69	0.311
4	2/ 1/79–15/ 1/79	15.41	11.01	0.15	0.200
5	24/ 1/79– 6/ 2/79	13.69	8.14	0.74	0.076
6	13/ 2/79–26/ 2/79	6.12	1.55	0.19	0.129
7	6/ 3/79[b]	27.69	19.58	12.63	0.091

[a] Dates given as day/month/year
[b] Single trapping

and from homozygosis of the mutations in field-reared, nontranslocation males and females (Whitten et al. 1977; Whitten 1979; Foster et al. 1985, 1988).

In the present paper we use an analytical version of the simulation program GENCON (Foster et al. 1988) to examine the data obtained during the 1976–79 field experiments. Starting with the frequencies of mating of wild females by released males, estimated during the trials, the program predicts the incidence of descendants of released males, the rate of genetic death and its effect on population size under various ecological or genetic constraints. The results suggest that immigration of wild flies into the test areas probably contributed substantially to the failure to achieve suppression. However, immigration alone does not explain all the differences between observed and expected results.

Materials and methods

Genetic mutations and strains

The *L. cuprina* mutations mentioned in the present report are: white eye (w) on chromosome 3, and topaz eye (to) on chromosome 5 (Foster et al. 1981; Maddern et al. 1986). The sex-linked translocation T(Y;5;3)23-1 has been described previously (Foster et al. 1980, 1985).

Estimates of genetic and ecological parameters from field-trial data

Field trials. Ecological and genetic data from the field trials conducted in New South Wales at Wee jasper (1976–78) and near Boorowa (1978–79) (Foster et al. 1985; Vogt et al. 1985) are summarized in Table 1. Ecological data (population densities) from the nonrelease areas and genetic data (frequencies of matings by released males) from the release areas were used in all simulations.

The genetic parameters used for simulation of the Wee Jasper trial were estimated from the progeny tests of field-inseminated females captured in traps 1–10 (details in Foster et al. 1985; Vogt et al. 1985). Prior to November 1977, flies from these traps were pooled before sampling for progeny testing. After this date (i.e., from generation 10 onwards), flies from trap 1 and traps 2–10 were progeny tested separately. For the purposes of the present analysis, the data obtained from November 1977 onwards were combined by weighting the genetic test results according to the number of females trapped in each trap group.

For simulation of the Boorowa trial, only data from the main release zone (area C of Foster et al. 1985) were used. No simulations were attempted using data from the barrier zones (areas B, D), since there were insufficient data from each zone during the first generation, and data from the two zones could not be pooled because they were heterogeneous [using the sea-

Table 2. Mating-type categories[a]

Male parent genotype	Female parent genotype			
	+/+;+/+	w/+;to/+	w/+;+/+	+/+;to/+
+/+;+/+	A	B	C	D
w/+;to/+	B	E	F	G
w/+;+/+	C	F	F	B
+/+;to/+	D	G	B	G
T(Y;5;3)/w;to	H	J	L	M
T(Y;5;3)/+;+	I	K	R	S
T(Y;5;3)/w;+	P	L	L	O
T(Y;5;3)/+;to	Q	M	N	M

[a] After Foster et al. 1985

son totals of the mating types from these areas given in Table 9 of Foster et al. 1985, the heterogeneity G-statistic (Sokal and Rohlf 1969) = 10.07 (2 *df*), giving $P < 0.01$].

Mating types and genotype frequencies. Nineteen mating types were distinguished by progeny testing of field-caught females (Foster et al. 1985). These types (A through S) are as defined in Table 2. The frequencies of matings by released males (Table 1) were estimated as the sum of mating types H, J, and L to Q inclusive, which is the sum of the frequencies of matings by T(Y;5;3)/w;to, T(Y;5;3)/w;+, and T(Y;5;3)/+; to males. The latter two genotypes were rarely generated in the field trials and were therefore assumed to have arisen by recombination in the mass-rearing colony (Foster et al. 1980, 1985).

The frequencies of matings by field-reared translocation males were those estimated for T(Y;5;3)/+;+ males (i.e., the sum of mating types I, K, R, and S).

The frequencies of field-reared mutation heterozygotes among nontranslocation (non-T) males and females were estimated as follows:

$$\text{frequency} = Y/Z,$$

where Y = the sum of the frequencies of mating types B to D inclusive, J to O inclusive, and R and S, plus twice the sum of the frequencies of mating types E to G inclusive; and where Z = twice the sum of mating types A to G inclusive, plus the sum of mating types H to S inclusive.

Genetic death. Estimates of the total rates of genetic death each generation during the trials (TGD) were derived from the progeny test results as follows:

$$D_t = (1.0 - (F_t \cdot P_f / 0.5)) \cdot (M_t / N_{ft})$$

$$D_m = 0.75\,(1 - D_t) \cdot f_J + 0.5\,(1 - D_t) \cdot f_{LM} + 0.4375\, f_E + 0.25\, f_{FG}$$

$$\text{TGD} = D_t + D_m,$$

where D_t and D_m are the death rates due to the translocation (combined effects of semisterility due to aneuploid sperm and of distortion of the sex ratio in favor of males) and homozygosis of mutations, respectively; F_t is the fertility of the translocation (0.4537), based on the number of euploid offspring (male + female) reared from eggs ($N = 22{,}986$) laid by females mated to T(Y;5;3)23-1 males, divided by the number of such offspring from eggs ($N = 24{,}792$) laid by females mated to nontranslocation males; P_f is proportion of females (0.4604) among euploid offspring ($N = 7762$) in translocation male matings, and 0.5 is the proportion of female offspring in nontranslocation matings; M_t is the number of translocation matings in the progeny tests and N_{ft} is the number of females tested; f_J, f_{LM}, f_E, and f_{FG} are the frequencies of mating types J, (L + M), E, and (F + G), respectively, and 0.75, 0.5, 0.4375, and 0.25 are the proportions of mutant homozygotes expected from the four preceding mating types, respectively.

The fertility value for T(Y;5;3)23-1 given above differs from that given in Foster et al. (1985), because that value was based on female offspring only, and thus included the effects of semisterility and sex-ratio distortion in the one figure. The fertility given by Foster et al. (1985) is the equivalent of the value (1-TD), which from the above data is estimated here at 0.4178, slightly higher than their estimate of 0.403.

Estimation of generation times. The start of the first generation each spring was assumed to be the first trapping date on which flies were recovered. The durations of the first two generations in each trial were estimated, using genetic data on recovery of field-reared descendants of released males (Foster et al. 1985). From the third generation onwards, median emergence times were estimated using the method of Vogt et al. (1985), modified as outlined below. Generations were assumed to end halfway between each median emergence date until the beginning of April, at which time larvae entering the soil in the Canberra region begin the overwintering phase (Dallwitz and Wardhaugh 1984). Adult flies trapped from April onwards were considered to represent the final generation of the season. Generation times estimated using this method are given in Table 1. This procedure reduced the estimated number of generations to eight per season, compared to the nine and ten generations estimated in 1976–77 and 1977–78, respectively, by Vogt et al. (1985).

Population density estimates. Average population densities each generation (Table 1) were calculated as geometric means of the weekly standardized female trap catches (Vogt et al. 1983) obtained during each generation period.

Rates of population increase. The rates of population increase (RATINC) used in the simulations were those estimated from trap data in the nonrelease comparison areas (Vogt et al. 1985). RATINC values (Table 1) were calculated for each generation (G_i) by dividing the mean standard catch for generation G_{i+1} by the mean standard catch for generation G_i. These RATINC (G_i) values estimate the natural rate of population increase in the absence of genetic death. They reflect the reproductive potential under prevailing environmental conditions of the previous generation (number of female offspring per breeding female in generation G_{i-1}).

Simulation experiments

In all simulations the starting test populations consisted of 10^6 wild-type individuals of each sex. Several different simulations were performed on each data set. The simulations are summarized in Table 3, but are described in detail in Results.

In simulations involving adjustment of genetic parameters such as translocation sterility, the same adjusted value was used for each generation. However, in simulations in which seasonal (i.e., environmental) factors could have influenced results (heterozygote or translocation survival), adjustments were made each generation. Specifically, the adjustments were made to minimize the difference between observed (progeny test) genetic results and those predicted by the simulation from the results of the previous generation.

In simulations involving migration, if the predicted frequency of matings by field-reared translocation (T/+;+) males exceeded the observed frequencies, the rate of immigration of wild-type flies was adjusted until the predicted and observed proportions were equal. The predicted heterozygote frequencies

Table 3. Summary of simulation experiments

Simulation	Translocation sterility	Descendant survival		Migration		Density dependence
		T/+; +	Mutant-het	♂=♀	♀ only	
I	0.546	1.00	1.00	0	0	No
II	0.848	1.00	1.00	0	0	No
III	0.546	1.00	variable	0	0	No
IV	0.546	variable	1.00	0	0	No
V	0.546	1.00	1.00	variable	0	No
VI	0.546	1.00	1.00	0	variable	No
VII	0.546	1.00	1.00	variable[a]	0	No
VIII	0.546	1.00	1.00	0	variable[a]	No
IX	0.546	1.00	1.00	variable	0	Yes
X	0.546	1.00	1.00	0	variable	Yes

[a] External source of immigrants initially threefold larger than test population

and population sizes were then compared with those estimated from the field-trial data. If the observed frequencies exceeded the predicted frequencies, no adjustments were made.

The analytical program GC2

General description. Program GC2 is a modification of program GC1 (Foster et al. 1988), designed to facilitate analysis of genetic data obtained during field trials. The necessary modifications have been made to the main program, with the genetic subroutines PSPERM, PROVA, ZYGOTE, and GENEXT remaining identical for the two programs. The program is run interactively, one generation at a time. Each generation, the operator has the option of adjusting one or more of the ecological parameters (chosen at the start of each simulation), in order to minimize the difference between observed and predicted frequencies of a selected genotype/mating-type category. At the end of the simulation, predicted population trends and the frequencies of other genotypes may be compared with those estimated from data obtained during the trial.

Calculation of release numbers. Release numbers used in the simulations were based on the effective release rate, i.e., the actual frequencies of matings by released males (PREL), estimated from the progeny tests of females trapped during the field trials as follows:

$$\text{PREL} = M_r / N_{ft},$$

where M_r = the sum of the matings by released males, and N_{ft} = number of females tested.

The numbers released in the simulation experiments (REL) were calculated each generation after immigration/emigration or adjustment for translocation and/or heterozygote survival, as follows:

$$\begin{aligned} N_{fr} &= \text{PREL} \cdot N_{bf} \\ P_{fr} &= N_{fr} / N_{mf} \\ A &= M - M_{tm} \\ B &= (P_{fr} \cdot A)/(1.0 - P_{fr}) \\ \text{REL} &= B - M_{tm}, \end{aligned}$$

where N_{fr} = number of females in the population mated by released males, N_{bf} = number of females in the breeding population (i.e., females that mated locally + immigrant mated females), P_{fr} = proportion of locally mated females mated by released T/mutation males, N_{mf} = number of locally mated females, M = number of field-reared males (local + immigrant), and M_{tm} = number of field-reared T/mutation males generated in the previous generation by the program. Released males calculated thus were added to the T/w;to males generated by the program.

Genetic death. The simulated genetic death (SGD) calculations used in GC2 are identical to those used in program GC1, with the addition of a term to include the genetic death caused by lowered survival of heterozygote offspring of released males, where this parameter was adjusted in the simulations. Note, however, that in Foster et al. (1988), the calculations performed in GC1 were described incorrectly. *Except for the HD term, the calculations performed in GC1 were as described below for GC2.*

The calculation of total population genetic death each generation in GC2 was as follows:

$$\begin{aligned} \text{TD} &= (1.0 - (F_t \cdot P_f / 0.5)) \cdot P_{tm} \\ N_T &= N_v + N_m + N_h \\ \text{MD} &= N_m / N_T \\ \text{HD} &= N_h / N_T \\ \text{SGD} &= (\text{TD} + (\text{MD} + \text{HD}) \cdot (1.0 - \text{TD})) \cdot N_{mf} / N_{bf}, \end{aligned}$$

where TD, MD, and HD are translocation death, mutational (homozygote) death, and heterozygote death, respectively; F_t, P_f, N_{mf}, and N_{bf} are as defined above; P_{tm} = proportion of locally mated females mated by translocation-bearing males; N_v = viable (homozygous wild-type + surviving heterozygotes) female offspring; N_m = mutant (all inviable) female offspring; N_h = the inviable heterozygote female offspring; and N_T = the total number of female offspring.

Competitiveness. Competitiveness of the released males that mated with wild females was assumed to be 1.00. Competitiveness of field-reared descendants of released males (either T/+; + males or nontranslocation mutation heterozygotes) is included in the survival parameter for these genotypes, which is set at 1.00 except in simulations III and IV (Table 3).

Migration. Migration during a given generation was simulated by discarding from the test population a certain proportion of the individuals of each genotype, and adding to the test population the same proportion of the wild-type individuals from a hypothetical external population not subjected to genetic control (Foster et al. 1988). Immigrant females were assumed to have already mated and were added to locally mated females to form the breeding (or ovipositing) population. Immigrant males were added to the population prior to mating (in the simulations, females are assumed to mate only once). The initial size of the external population was set at either equal to that of the test population or at some multiple of the initial test population. After generation 1, the sizes of the external populations were controlled by the RATINC values estimated in nonrelease areas.

Results

Observed and predicted genetic and population trends

The frequencies of matings by released males and the rates of genetic death estimated by progeny testing of trapped females at Wee Jasper and Boorowa (Foster et al. 1985) are summarized in Fig. 1 a. Each season, the frequency of matings by released males declined in midsummer (generations 4–6 and 11–14 at Wee Jasper, and generations 5–7 at Boorowa) compared to springtime levels, coinciding with the reduced survival of released larvae during the hotter summer months (Vogt et al. 1985). Genetic death declined more slowly than matings by released males following the springtime peaks. Presumably, field-reared translocation males and heterozygous females (descended from the released males) were contributing substantially to the genetic death (Foster et al. 1985, 1988). This in turn suggests that the descendants had not been subjected to the same harsh environmental conditions as the released males, but enjoyed conditions similar to those experienced by native *L. cuprina* (Vogt et al. 1985).

Population densities estimated during the Wee Jasper and Boorowa trials are summarized in Fig. 1 b. It is clear that despite the occurrence of substantial genetic death, population sizes did not decrease in the release areas relative to those in the nonrelease areas. In both trials, midsummer declines were greater in the nonrelease areas than in the release areas. These differences may reflect local differences in sheep-carrying capacity and weather patterns, and underscore the difficulty of using such comparison areas to monitor the effectiveness of genetic control trials, without several years of prerelease ecological data (Vogt et al. 1985).

Simulations assuming no migration and full survival/competitiveness of descendants of released males (type I, Table 3) gave both genetic and ecological predictions (Fig. 2) which diverged widely from the observed trends. These simulations predicted that final population sizes should have been approximately 50-fold lower than without releases in the Wee Jasper trial, and 12-fold lower in the Boorowa trial (Fig. 2 a). Moreover, the observed frequencies of both field-reared T/+;+ males and nontranslocation heterozygotes were considerably lower than predicted (Fig. 2 b, c). In order words, the frequencies of genotypically wild-type flies and of matings by nontranslocation males were substantially higher than expected. Thus, although the observed rates of genetic death suggested better survival of descendants of released males than of released males themselves (Fig. 1 a), the observed frequencies of these genotypes were much lower than expected.

Of the many possible explanations of the differences between predicted and observed results, the simplest

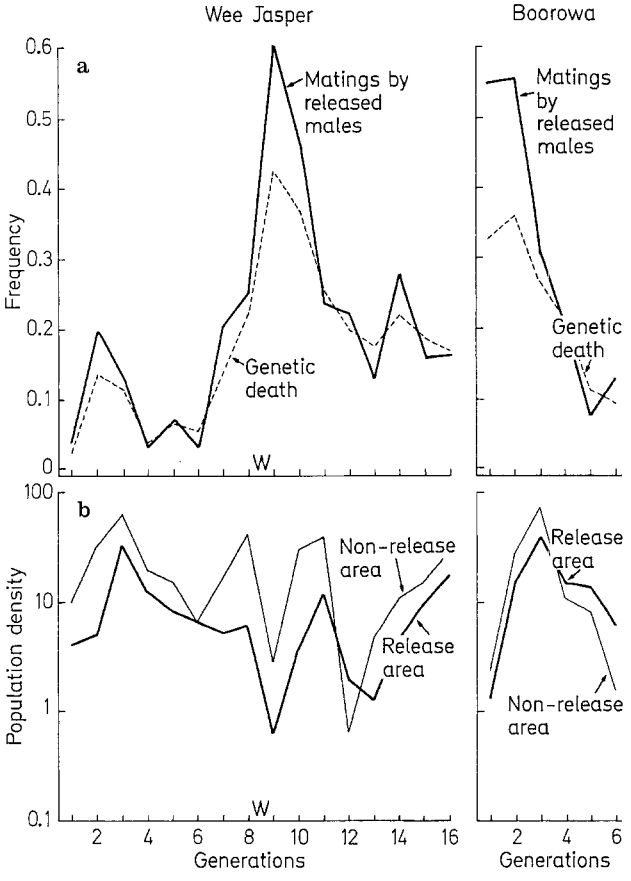

Fig. 1 a and b. Genetic and ecological trends in the Wee Jasper and Boorowa field trials. **a** Estimated frequencies of matings by released males and rates of genetic death (TGD). **b** Population densities in nonrelease areas and release areas. Densities are expressed as standardized catch/trap/hour (Vogt et al. 1983)

would be incorrect estimates of genetic parameters, such as translocation sterility or survival of particular genotypes, or incorrect ecological assumptions, such as isolation of target populations or density dependence. These possibilities were explored using the simulations described below.

Translocation sterility

If the sterility used for the translocation was an underestimate of the actual value, both the heterozygote frequency and the frequency of field-reared translocation males would be overestimated by the simulation. However, simulations in which higher translocation sterility levels were assumed did not result in consistently more accurate predictions. In the example shown (simulation II, Table 3), a sterility set as high as 85% still predicted population sizes (Fig. 3 a) similar to those predicted by simulations using the translocation sterility level of 55% (Fig. 2 a). Predicted T/+;+ frequencies were similar to those observed at Boorowa but substantially lower than those observed at Wee Jasper (Fig. 3 b). Predicted het-

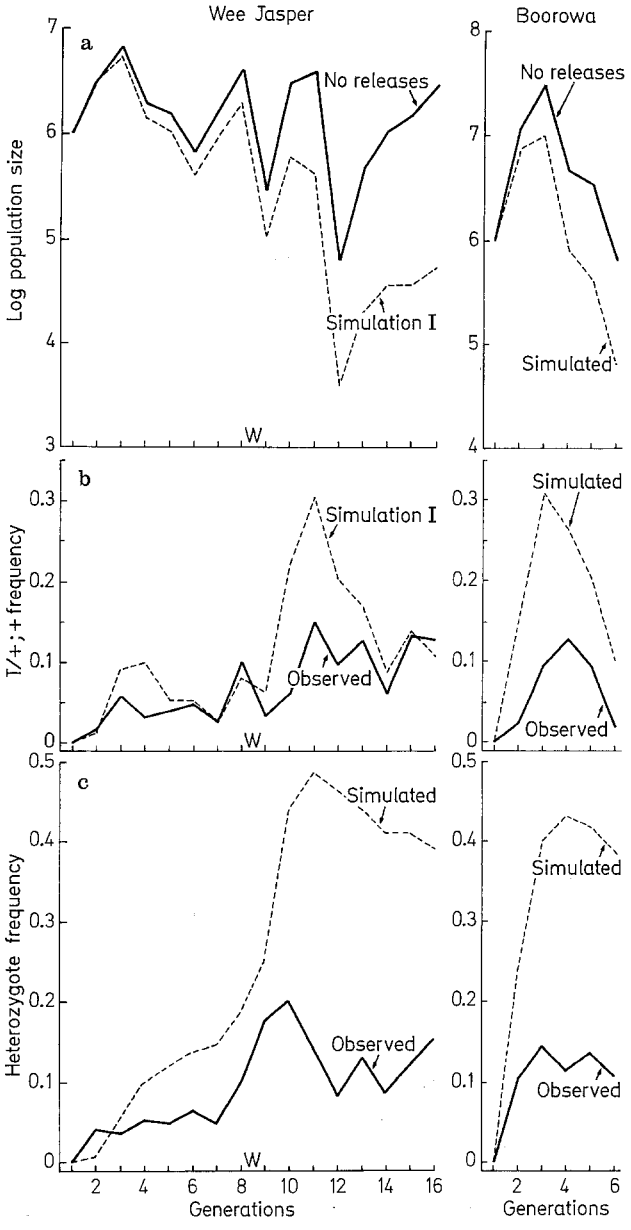

Fig. 2a–c. Simulations of genetic control with full survival of field-reared descendants of released males and no immigration (simulation I, Table 3). **a** Population trends, **b** T/+;+ frequencies, **c** mutation heterozygote frequencies. *W* indicates winter

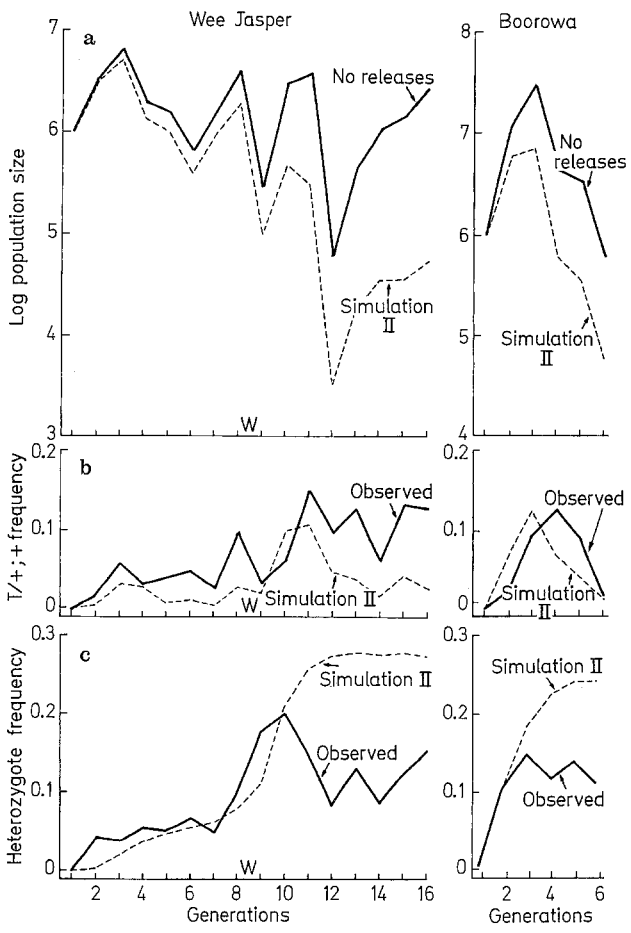

Fig. 3a–c. Simulations assuming 86% sterility of released males (simulation II, Table 3). **a** Population trends, **b** T/+;+ frequencies, **c** mutation heterozygote frequencies. *W* indicates winter

erozygote frequencies were close to the observed values, particularly during the first half of the Wee Jasper trial, but not during the second half of this trial or during the Boorowa trial (Fig. 3c).

Survival/competitiveness of different genotypes

Lower survival/competitiveness in the field of T/+;+ or mutant heterozygote genotypes could possibly account for the differences between observed and predicted results. For example, the chromosome rearrangement carried by such males could have a partially dominant deleterious effect under some environmental circumstances, as could eye-color mutations carried in the heterozygous condition. However, simulations in which survival/competitiveness of either heterozygotes or T/+;+ males was reduced (simulations III, IV, respectively, Table 3), suggest that neither mechanism can explain all the results (Fig. 4).

Adjusting the survival of field-reared translocation (T/+;+) males (simulation IV, Fig. 4d) to generate the observed frequencies of this genotype each generation, resulted in only slight changes to predicted population sizes (higher) and heterozygote frequencies (lower) (Fig. 4a, c), compared to simulation without adjustments (Fig. 2a, c). Fitting the survival of heterozygotes (simulation III, Fig. 4d) predicted even greater population suppression (Fig. 4a) (because reduced heterozygote survival is equivalent to increased genetic death) and higher T/+;+ frequencies (Fig. 4b) than simulation without adjustments (Fig. 2a, b). Another simulation (not shown in Table 3), in which survival of both T/+;+ and het-

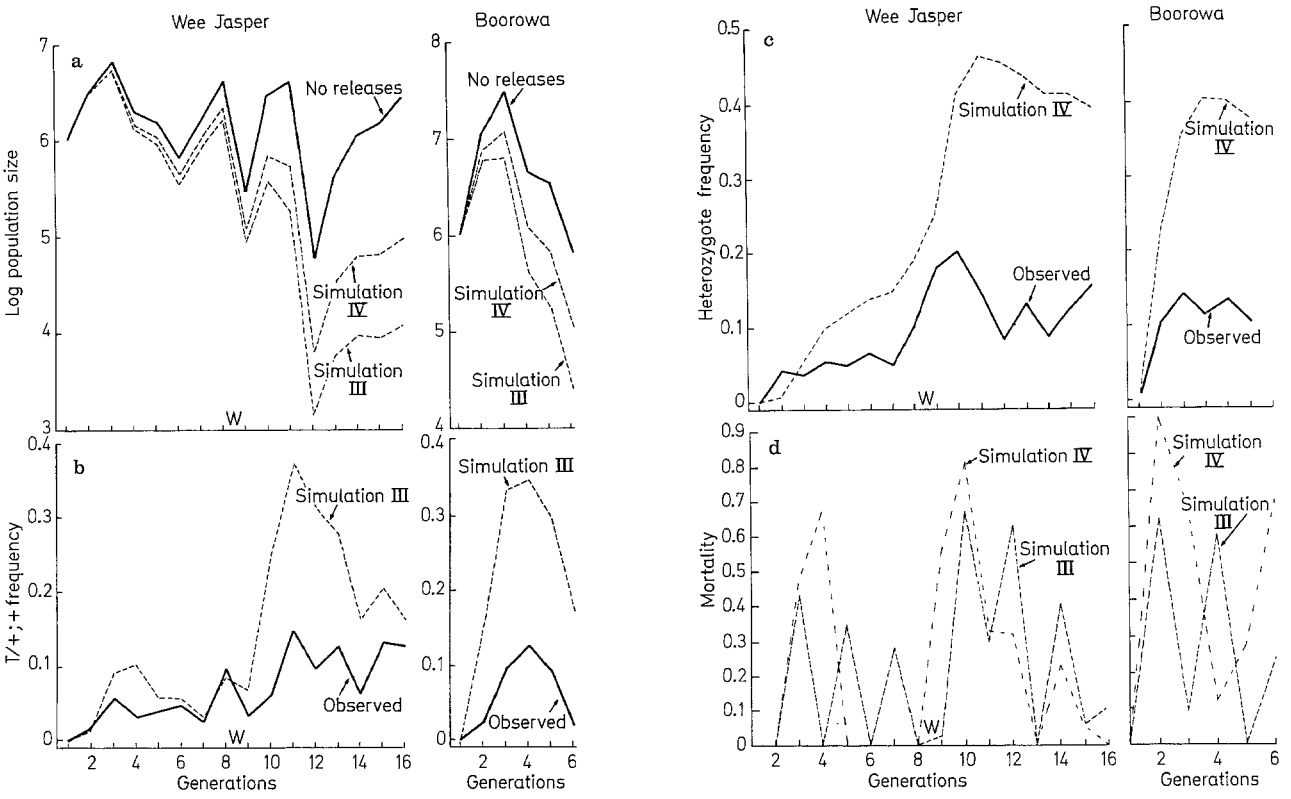

Fig. 4a–d. Simulations involving reduced survival of descendant genotypes. **a** Population trends (simulations III and IV, Table 3). **b** Predicted T/+;+ frequencies with reduced heterozygote survival (simulation III). **c** Predicted heterozygote frequencies with reduced T/+;+ survival (simulation IV). **d** Heterozygote and translocation mortalities. *W* indicates winter

erozygotes was reduced, predicted population sizes lower than those of simulation III.

Immigration of wild flies

Migration of wild flies into the release area would be expected, both to reduce the frequencies of heterozygotes and field-reared translocation males and to increase the size of the test population. Indeed, the simulations in which migration was adjusted each generation to fit the observed frequency of T/+;+ males gave a better fit between predicted and observed heterozygote frequencies and population trends than any of the previous simulations (Fig. 5). Migration of equal numbers of both sexes (simulation V, Table 3) predicted heterozygote frequencies more accurately in the Boorowa trial, whereas migration of mated females but no males (simulation VI) was more accurate in the Wee Jasper trial.

The population sizes predicted with these simulations (Fig. 5) were closer to the nonrelease simulation than those predicted in simulations I–IV (no migration) (Figs. 2–4). However, these simulations still tended to predict greater population suppression than in the nonrelease simulation, especially with the Wee Japser data. Predicted final population sizes at Wee Jasper were eightfold lower (migration of both sexes) and fivefold lower (females only) than those in the absence of releases (Fig. 5a).

If the external source of immigrants were larger than the target population, removal of fewer flies from the test population and addition of more wild-type flies from the external population would be required to achieve a given fit to the genetic data. This would result in identical heterozygote frequencies to those predicted in simulation V, but increased population sizes. Simulations with the external population initially three times the size of the test population (simulations VII, VIII) did give better agreement between predicted and observed population size (Fig. 6). In the Boorowa simulations, final predicted population sizes were very close to the nonrelease simulation. In the Wee Jasper simulations, predicted population sizes tended to be slightly overestimated in the 1st year of the trial and underestimated in the 2nd year. Final predicted population sizes in the Wee Jasper simulations were two- and 4.4-fold less for the females-only and both-sexes immigration cases, respectively (Fig. 6).

The simulated levels of migration required to achieve the best fits to the genetic data are summarized in Fig. 7. The rates of migration thus estimated were sometimes high and were not uniform each generation. In the Wee Jasper trial simulations, estimated migration between populations was highest in the first half of each season,

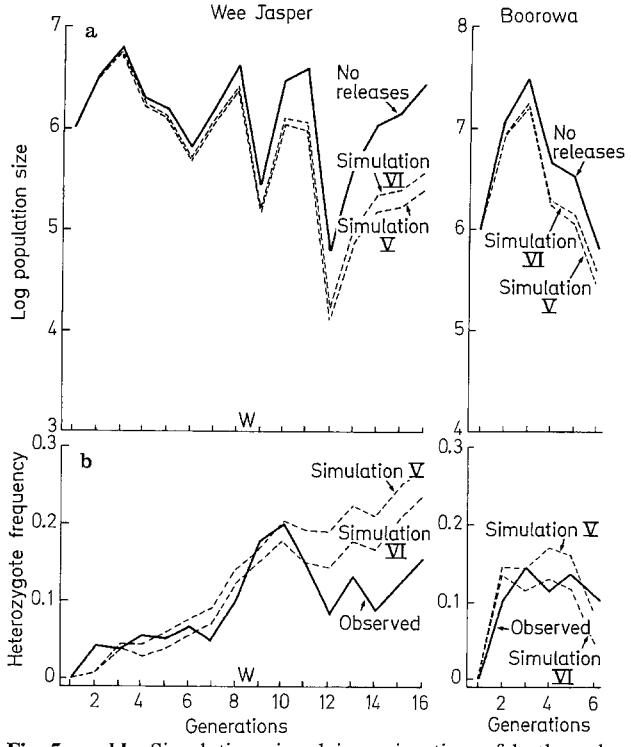

Fig. 5a and b. Simulations involving migration of both males and mated females (simulation V, Table 3) or of mated females only (simulation VI), with initial external population equal in size to test population. **a** Population trends, **b** heterozygote frequencies. *W* indicates winter

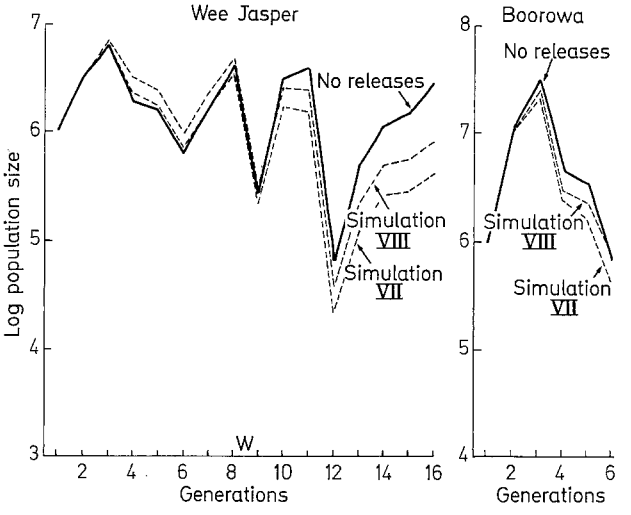

Fig. 6. Population trends in simulations involving migration with initial external population threefold larger than test population (simulations VII and VIII, Table 3). *W* indicates winter

declining considerably in the second half of each season. The Boorowa trial simulations predicted similar rates of migration during the first half of the season, but this trial was terminated before any trend for the second half of the season could be determined.

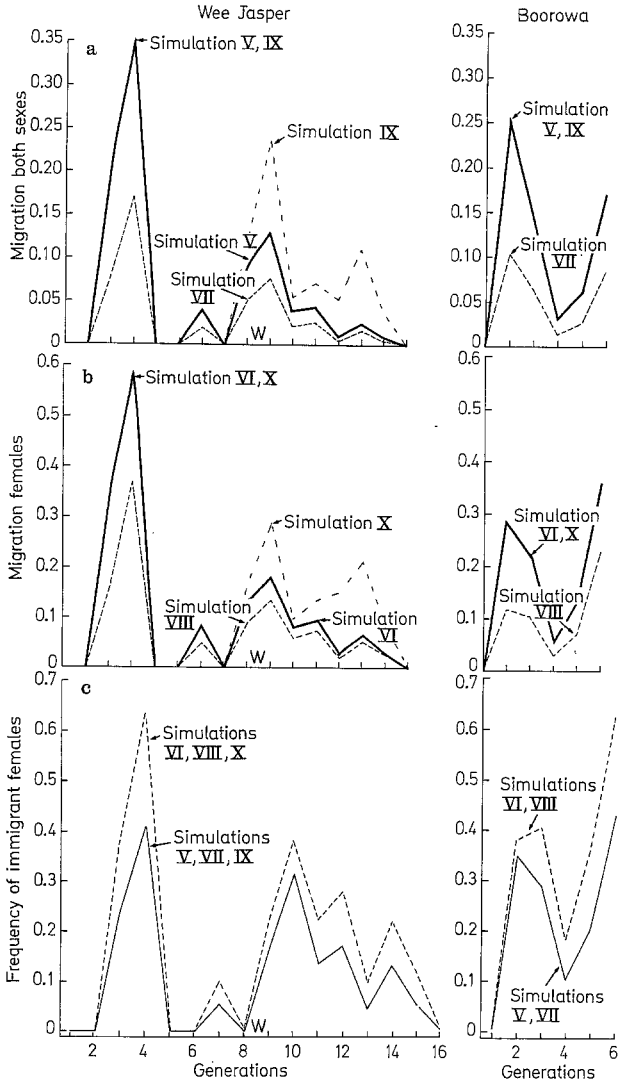

Fig. 7a–c. Estimates of migration. **a** Proportions of external population (both sexes) transferred to test populations (simulations V, VII, and IX, Table 3). **b** Proportions of external population (mated females only) transferred to test populations (simulations VI, VIII, and X). **c** Proportions of immigrant females in the test populations. *W* indicates winter

Lower migration rates (expressed as proportions of populations) were required when both sexes were transferred into test populations (Fig. 7a) than when mated females only were allowed to migrate (Fig. 7b). With the external population larger than the test population, lower rates of movement were required (although the actual numbers transferred were greater) than with equal population sizes.

The estimated proportions of immigrant females in the test populations (Fig. 7c) are of more interest from the practical point of view than rates rates of exchange between hypothetical populations. These are effectively estimates of the proportion of mated females originating

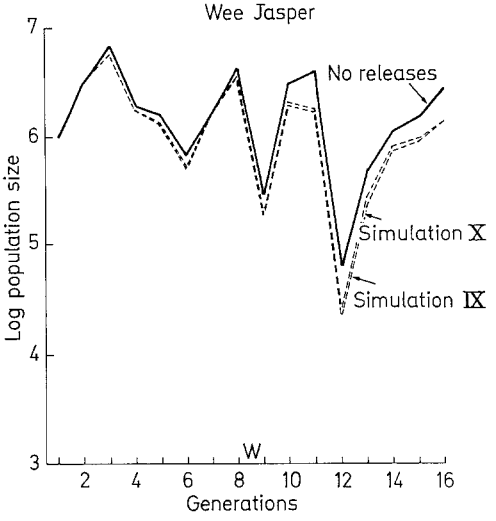

Fig. 8. Population trends in simulations involving migration of both males and mated females (simulation IX, Table 3) or of mated females only (simulation X), with density-influenced population regulation

outside the test area, and are the same regardless of the external population size.

Density dependence and immigration

The failure of simulations that involved only immigration to adequately predict density trends at Wee Jasper could reflect the lack of allowance for effects of population density on the rates of population increase. To examine this possibility, simulations of the Wee Jasper trial were performed in which both migration occurred and rates of increase were increased at lower densities. In simulations IX and X (Table 3), RATINC values were multiplied by a density factor DF, when the size of the population was below 10^6 ovipositing females. This factor was calculated after Prout's (1978) formulation:

$$DF = DF_{max} \cdot K/(K + (DF_{max} - 1) \cdot N),$$

where DF_{max} = the maximum density factor; $K = 10^6$, the population size below which DF is greater than 1.0 (analogous to Prout's equilibrium population density); and N is the size of the breeding population (number of ovipositing females).

Using external populations equal in starting size to the test population, and with $DF_{max} = 2.0$, population size predictions for the Wee Jasper simulations were improved considerably, the final population sizes being only twofold less than those predicted by the nonrelease simulations (Fig. 8). Predicted heterozygote frequencies were virtually identical to those shown in Fig. 5b. Predicted population trends for Boorowa (not shown) were not improved by these simulations over those shown in Fig. 5a, because the predicted population sizes exceeded 10^6 females for all generations except the last one. The migration rates required to fit the genetic data increased only when DF was greater than 1.0, i.e., during the second season of the Wee Jasper trial (simulations IX and X, Fig. 7a, b).

Discussion

Simulations involving reducing the fertility of released males or varying survival of field-reared translocation males or mutation heterozygotes, to achieve a good fit to genetic data on mating frequencies by released males, do not provide good fits to the remaining genetic data or to the ecological data. If the survival of both T/+;+ males and heterozygotes were reduced, an excellent fit to all the genetic data could be achieved, but this predicted even greater population suppression than the no-migration, 100% survival case (Fig. 2).

Simulations of genetic control with migration suggest that immigration of wild flies could be a sufficient explanation of the failure of the releases at Boorowa to achieve suppression, and could at least be a partial explanation in the case of Wee Jasper.

The final population sizes (i.e., number of ovipositing females) predicted when immigrants were restricted to females were higher than when immigration of both sexes was allowed, because the former procedure introduces less wild-type genetic material to the population and therefore requires a greater immigration rate to achieve a fit to the genetic data. Although immigration of females only provided the best fit to the observed population trends, there is considerable evidence that males can migrate also (Wardhaugh et al. 1983; Foster et al. 1985; G. G. Foster, W. G. Vogt, K. G. Wardhaugh, M. J. Whitten and T. L. Woodburn, unpublished results).

Simulations involving immigration alone predicted smaller final population sizes than were observed, particularly at Wee Jasper. This suggests that one or more of the assumptions underlying those simulations needs to be modified. The migration simulations assumed equal rates of migration of locally bred flies from the test population and of immigrants from the hypothetical external population. If the rate of emigration from the test area were less than the rate of immigration, a higher final population size would have been predicted. The extreme case of no emigration from the test area, coupled with significant immigration from nonrelease areas, is not tenable, as Foster et al. (1985) provided direct evidence of emigration from the Boorowa release area.

Despite the failure of simulations of reduced translocation survival to account for the observed genetic and population data (Fig. 4), it does seem likely that this probably did contribute in some measure to the lower than expected frequency of T/+;+ males. The males

released in these trials carried a dieldrin resistance (*Rdl*) allele on the translocation complex, originally intended as a genetic sexing measure (Whitten and Foster 1975; Foster et al. 1978, 1985). McKenzie (1990) has recently reported that in the field, *Rdl*/+ heterozygotes are at a slight disadvantage compared to +/+ during most of the season (9% mortality), but that the disadvantage is severe in winter (89% mortality). Thus, particularly in generation 9 (the emerging generation after the winter period) of the Wee Jasper trial, the difference between observed and expected T/+;+ frequency may have been due mainly to this cause. Simulation IV predicted high (55%) translocation mortality in this generation (Fig. 4d). However, similarly high translocation mortality predicted at several other (warm-weather) times during this and the Boorowa trial cannot be attributed to the effects of the *Rdl* allele. Finally, low fitness of the *Rdl* gene cannot explain the low frequencies of eye-mutation heterozygotes, which carried the wild-type allele at this locus.

Another implicit assumption is that the population trends, estimated from the ecological studies of the nonrelease areas, are applicable to release areas. Vogt et al. (1985) concluded that this was almost certainly not the case in the Wee Jasper trial. Even where this assumption is valid, such rates of increase must include any effects related to population density. In *L. cuprina* populations, density-dependent increase is probably not related to competition by larvae for limited resources (Dallwitz 1987), but to the response of farmers to increased rates of infestation of sheep (Foster et al. 1975).

If the effect of genetic control methods were to lower the infestation rate, causing farmers to reduce the frequency of insecticidal treatment of sheep below that employed in the nonrelease areas, any increase in the ability of such populations to recover from these effects could not be estimated from the available data. Thus, the failure of the simulations to predict the observed population trends could reflect a degree of success of the treatment that is not measurable by other means (such as density estimates from trap catches).

At densities near and below a standardized catch rate of 1 female/trap/hr, the rate and extent of sheep infestation would not generally be perceived as a serious problem by graziers (Wardhaugh and Morton 1990). Under these circumstances, the frequency of preventative measures such as insecticidal application would be likely to decrease, leading to improved chances for larval survival on sheep (McKenzie and Whitten 1982), and thus to a density-related increase in population fecundity (Foster et al. 1975).

Comparing the population trends at Wee Jasper predicted in the migration simulations (Figs. 5a and 6) with the observed starting densities at Wee Jasper (Fig. 1b) suggests that the predicted standardized catch rates would, indeed, have been in the range of 1 female/trap/hour or less for much of the 2nd year of the trial (generations 9–16). Thus, it does not seem unreasonable to invoke density effects to partially explain the lack of correspondence between observed and predicted populations trends, although immigration would still appear to be the major cause. The identical comparison for the Boorowa trial simulations suggests that predicted population sizes would have been within this range only during the hot summer months, when fly attack would not have been a serious problem anyway.

The average estimated proportions of immigrant females in the trial areas were 9% for Wee Jasper and 21% for Boorowa. These estimates are consistent with the relative isolation of the Wee Jasper area compared to the Boorowa area. The former release area is bounded on two sides by rugged wooded ranges and on a third side by the Burrinjuck Reservoir, whereas the Boorowa release area is surrounded by open, flat country uniformly stocked with sheep (Vogt et al. 1985). Clearly, the 5-km wide "barrier" zone of released males in the latter trial was not adequate to buffer the main release area against wild-type gene flow from outside (cf. Wardhaugh et al. 1983). Although independent estimates of immigration were not possible from the field-trial data, an indication of fly movement in the Boorowa trial can be inferred from the observation that 13% of females mated by field-reared (T/+;+) males were recovered more than 2 km outside the release zone (Foster et al. 1985). This figure is of the same order as the immigrant frequency estimated using simulations.

Although the estimated migration rates appear high, this reflects the relatively small dimensions of the test areas rather than high fly mobility. Large-area genetic control programs would only be subject to the effects of immigration at the edges of the release zones, since the rates of movement for *L. cuprina* appear generally to be low (Gilmour et al. 1946; Norris 1959; Foster et al. 1975), although individuals are capable of travelling considerable distances (Wardhaugh et al. 1983).

A final comment supporting the likely importance of immigration in the Wee Jasper and Boorowa trials comes from the results of a later trial conducted on an island 27 km off the coast of South Australia (R. J. Mahon and G. G. Foster, unpublished results). In this trial, in which natural immigration was negligible, mutation frequencies and population density approximated the expected levels, with the fly population becoming undetectable by the end of the release period.

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