Theor Appl Genet (1985) 70:13–21

Field trial of a compound chromosome strain for genetic control of the sheep blowfly *Lucilia cuprina*

G. G. Foster, R. H. Maddern, R. A. Helman and E. M. Reed
CSIRO Division of Entomology, G.P.O. Box 1700, Canberra, ACT 2601, Australia

Received November 16, 1984; Accepted November 30, 1984
Communicated by J. S. F. Barker

Summary. In 1979–80 a field trial of a compound chromosome (CC) strain of the Australian sheep blowfly *Lucilia cuprina* was conducted in the isolated Brindabella Valley, N.S.W. New genetic material was introduced into the strain before release by inducing 104 new CC elements by irradiation of recently captured field strains, and combining the resulting strains. Weekly releases, averaging 1.1 million larvae per week, were begun in November 1979 and continued to May 1980. Field-inseminated females were trapped weekly and their genotypes and those of their mates were determined through genetic testing. The proportion of wild × wild matings declined from 16% in December 1979 to 1% in April 1980. During this period the proportion of CC × CC matings rose from 50% to 90%. Larvae sampled from infested sheep had compound chromosomes, indicating that compound chromosome-bearing females can successfully oviposit in the field. Trapping of flies resumed at the start of the 1980–81 season, without further releases. Progeny tests revealed the presence of both CC and wild flies. The proportions of CC × CC matings among field-inseminated females were 90% in October, 44% in November, nil in December, and 12% in January. No CC × CC matings were detected in 33 field-inseminated females trapped and tested during April, and 70 tested males reared from myiasis samples in April 1981 proved to be wild type. These results indicate that the CC strain overwintered in the field and strongly suggest that it bred in the field for at least one generation following the spring emergence before being eliminated from the population.

Key words: Genetic control – Compound chromosomes – *Lucilia cuprina*

Introduction

The possibility of using chromosome rearrangements for genetic control of *Lucilia cuprina* (Wiedemann) has led to a considerable amount of research into its basic genetics (Foster et al. 1980b, 1981) and, more particularly, into the construction of compound chromosome (CC) strains in this species (Foster et al. 1976; Maddern 1981). In theory, a CC strain, if released in sufficient numbers, should be able to completely replace a wild population (Foster et al. 1972; Fitz-Earle et al. 1973). The results of a preliminary field trial in 1975–76 of the inbred CC strain *C(5L)2;C(5R)1* of *L. cuprina* suggested that this strain was poorly competitive under field conditions (Whitten et al. 1977; Foster 1980). In the present paper we report the results of a field trial using CC strains constructed by pooling a large number of independently isolated compound fifth-chromosomes.

Materials and methods

L. cuprina mutations and marked CC strains

The names and symbols of mutations mentioned in this report are as follows: black body (b^2) on the X chromosome, black puparium (*bp*) on chromosome 2, white eye (*w*) on chromosome 3, topaz eyes (*to*) on the left arm of chromosome 5, and stubby bristles (*sby*) on the right arm of chromosome 5.

C(5L)3,to (Maddern 1981) is a compound left fifth-chromosome, homozygous for *to*.

C(5R)2,sby (Maddern 1981) and *C(5R)5,sby* are compound right fifth-chromosomes, homozygous for *sby*.

Isolation of compound-fifth chromosomes and construction and properties of release strains

The CC strains for release were composite wild-type strains made by combining a number of strains carrying different *C(5L),+* and *C(5R),+* chromosomes. The CCs were generated

Table 1. A MR-line composition and number of independent compound chromosomes in each release strain

Strain	MR-lines used	No. of CCs in each strain	
		C(5L)	C(5R)
C17	1–7, 10, 12, 14, 15	20	43
C18	1, 3–6, 12–14, 19, 39	24	40
C20	41, 44, 47	10	12
C21	2, 8, 42, 45, 48	20	25

B Relatedness of the different release strains

Strains	No. of CCs exclusive to, or shared by different release strains	
	Left	Right
C17	0	4
C18	0	3
C20	3	9
C21	5	10
C17, C18	11	26
C17, C18, C21	7	10
C17, C21	2	3
C18, C21	1	0
C18, C20, C21	3	0
C20, C21	2	2
C18, C21	2	1
Total no. of independent CCs	36	68

Table 2. Fertility of marked compound strains and MR lines

Line	Total no. of*		Rank mean (%)**
	Eggs	Adults	
C(5L)3; C(5R)2	867	55	5.29[a]
C(5L)3; C(5R)2	1,893	172	6.46[a]
MR12	2,614	237	6.49[a]
MR3	2,676	216	7.61[a]
MR6	2,608	251	7.71[a]
MR4	5,259	516	7.73[a]
MR14	4,290	440	8.85[a]
MR13	4,194	467	9.50[a]
MR5	4,255	557	11.29[a,b]
MR1	2,581	404	15.81[b]
MR39	1,549	406	24.81[c]

* Pooled results from egg batches laid by single females. Data were excluded where a female produced less than 50 eggs or no hatch

** Mean % survival after angular transformation of data from single females. Rank was determined by Duncans multiple range test (Steel and Torrie 1960) after analysis of variance on the percent egg to adult survival transformed on an angular scale

[a,b,c] Strains not differing significantly at the 5% level have the same letter

Table 3. Mass rearing data and number of larvae released

Date		% Survival[a]	Estimated no. released (1,000's)	Strain released
Nov.	14 1979	10.0	1,300	C17
	21	10.2	920	C17
	28	8.7	740	C17
Dec.	5	6.6	1,050	C17
	12	6.6	1,070	C17
	19	7.2	920	C17
	26	8.9	1,560	C17
Jan.	2 1980	9.1	1,056	C17
	9	7.4	1,530	C18
	16	10.2	1,100	C18
	23	8.6	1,000	C18
	30	5.1	460	C18
Feb.	6	6.8	600	C18
	13	8.2	1,100	C18
	20	12.1	1,000	C18
	27	16.6	1,800	C18
Mar.	5	15.3	2,100	C18
	12	13.5	1,700	C18
	19	15.0	1,180	C18
	26	16.5	1,660	C18 + C20
Apr.	2	15.5	1,100	C18
	9	13.5	1,500	C18
	16	11.0	1,210	C20
	23	15.0	1,480	C21
	30	14.6	1,900	C18
May	7	10.7	1,000	C20
	14	15.7	2,000	C21
	21	15.2	2,800	C18

[a] Eggs to mature larvae

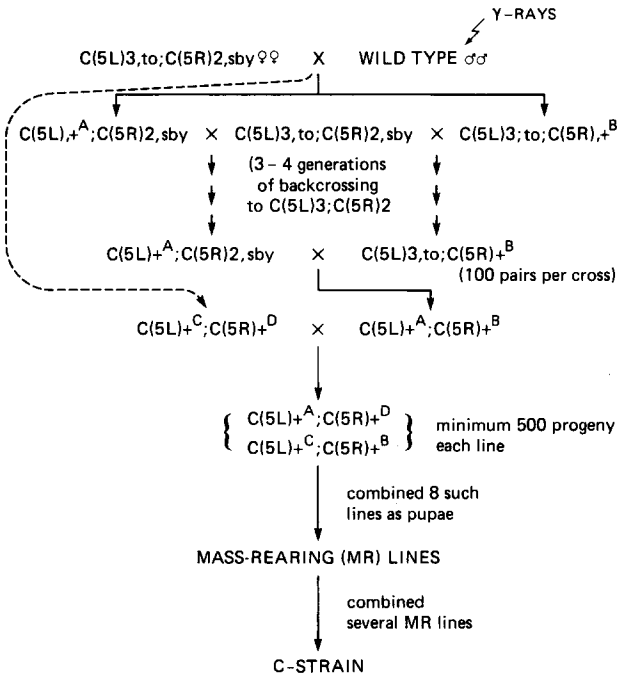

Fig. 1. Outline of the procedure used to construct compound-chromosome strains for release

by irradiating immature spermatocytes (Maddern 1981) in a series of wild strains collected in the Canberra region in 1978–79. Males were generally irradiated in the second or third generation after colonization from the field. The procedure used in isolating these CCs and combining them into the release strains is outlined in Fig. 1. In all, 36 *C(5L),+* and 68 *C(5R),+* elements were used (most of them in several mass-rearing (MR) lines/strains) in five different but related combinations. The composition of each strain is summarized in Table 1. Strains C17 and C18 were the most closely related to one another, while strains C17 and C20 were the least closely related.

Although from the viewpoint of minimizing inbreeding it would have been preferable to cross flies carrying a newly isolated *C(5L),+* with flies carrying a newly isolated *C(5R),+*, the low frequency of recovery of new putative CC isolates made this approach impracticable. Putative *C(5L),+* or *C(5R),+* elements were backcrossed to *C(5L)3,to; C(5R)2,sby* for 3–4 generations (sometimes more), firstly in order to verify the presence of a new CC, and secondly to obtain sufficient flies for further crosses. Fertility of this series of crosses was the principal determinant of the number of generations of backcrosses to *C(5L)3; C(5R)2* before the newly isolated CC was ultimately included in a release strain. Some rearrangements were lost at this stage because of low fecundity. Because of this procedure, the non-CC genetic background of the MR lines that were combined to make the release strains was similar to that in the marked strain *C(5L)3; C(5R)2* (Fig. 1). This strain in turn was related to the wild-type strain *C(5L)2; C(5R)1* used by Whitten et al. (1977), but contained additional genetic background, introduced by the induction of 20 new CCs in laboratory strains. One indication of this relatedness was the presence of substantial numbers (not actually counted) in *C(5L)3; C(5R)2* and the release strains, of individuals exhibiting the chromosome 2 mutation *bp*, which was homozygous in 13% of the flies in *C(5L)2; C(5R)1* (Whitten et al. 1977). Notwithstanding the similarity of genetic backgrounds, significant increases in fecundity compared to *C(5L)3; C(5R)2* were observed in some of the MR lines (Table 2).

Rearing and release details

Rearing procedures were similar to those described by Foster et al. (1980a) for the translocation strain *T23-1*, except that the larval diet consisted of ovine liver, meat meal, cotton linters and water in the following proportions by weight: 10 : 20 : 1 : 20. The total number of eggs laid was estimated by weighing, assuming an average of 18,000 eggs/g (unpublished data). The numbers of mature larvae produced were estimated from total weights, using weighed and counted subsamples from each larval harvest. Survival of the release strains from eggs to mature larvae was calculated for each rearing using these estimates (Table 3). Mature larvae were released aerially (Foster et al. 1978) at weekly intervals starting November 14, 1979, and ending May 21, 1980 (Table 3). No releases were made during 1980–81.

Release area

The release area was approximately 10 km² along the Goodradigbee River valley south of Brindabella, N.S.W. (altitude approximately 650 m), centered 39 km WSW of the Canberra City Post Office (Fig. 2). This area consists of improved and native pasture along the river flats and on nearby hills. The area is surrounded by rugged terrain and dense forest. At the time of the trial, the area contained approximately 1,200 sheep mainly on three properties. The nearest other property with sheep was approximately 17 km NNW of the northern end of the release area. No sheep were imported into the release area during the trial.

Trapping details

L. cuprina were trapped using modified Western Australian blowfly traps (Vogt and Havenstein 1974) baited with minced sheep liver and sodium sulphide solution. Regular trappings in the release area were made for two seasons (November 1979–April 1980, and October 1980–May 1981). Fresh baits were used for each trapping until March 1980. From March 3 onward, old baits were used, as these permitted recovery of more flies (Woodburn and Vogt 1982) for progeny testing.

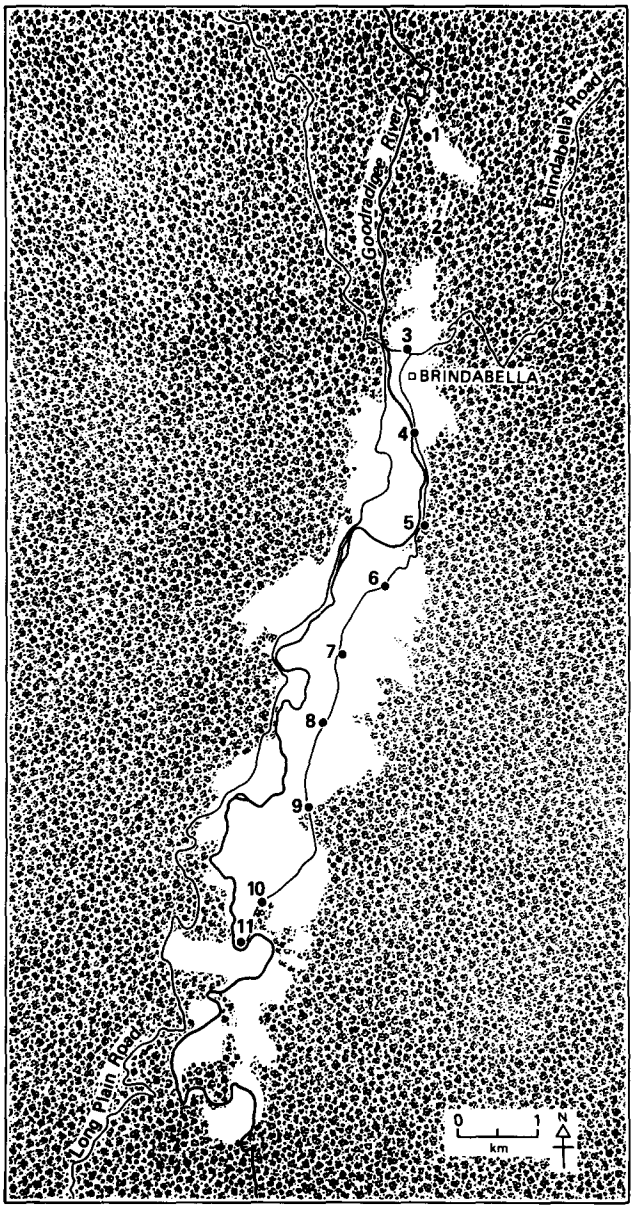

Fig. 2. Map of the Brindabella Valley and surrounding region. Numbers indicate trapsites

Table 4. Egg hatch data from laboratory crosses and progeny tests of field inseminated females

	Percent hatch (no. of egg masses in each hatch range)									
	1–4	5–10	11–20	21–30	31–40	41–50	51–60	61–80	81–90	91–100
A. Laboratory crosses										
Compound × Compound[a]	1	2	2	6	7	7				
[b]		5	13	17	26	3				
[c]	15	21	47	45	31	6	1			
Compound ♀ × Wild ♂ [a]	13	9	1							
Wild ♀ × Compound ♂ [a]	10	7	16	3						
Wild × Wild [a]	1		1		1		1		9	27
B. Progeny tests 1979–80										
Compound × Compound	7	25	79	113	87	19				
Presumptive hybrids	39	27	17	10	1	1				
Incomplete tests[d]	7	12	27	29	17	2				
Wild × Wild			2	1			1		11[e]	20
C. Progeny tests Oct., Nov. 1980[f]										
Compound × Compound	3	6	13	21	12	1				
Presumptive hybrids			1							
Incomplete tests		1	3	2	3					
Wild × Wild									2	20

[a] Combined data from three experiments (unpublished)
[b] Combined hatch data from Foster and Maddern (in preparation)
[c] Pooled data from the MR lines used in C18 (Table 2)
[d] See text
[e] Includes eight in which hatch was visually estimated as > 80%, but in which eggs were not counted
[f] Data from December onwards not usable because of toxic filter papers (see text)

Genetic and fertility tests of trapped flies

Egg masses obtained from individual field-inseminated females were spread onto moistened coloured blotting paper, incubated for 18 h at 25 °C, and then hatched and unhatched eggs were counted. If 50% or more of the eggs hatched, these were assumed to represent wild ♀ × wild ♂ (W × W) matings. Egg hatch counts of known compound × compound (C × C) matings nearly always yielded less than 50% hatch (Table 4 A).

Larvae from each egg mass which gave less than 50% hatch were reared in a single brood on sheep liver. Broods which gave no hatch were disregarded. Broods in which at least one egg hatched but which produced no adult offspring were presumed to have resulted from hybrid (C × W and W × C) matings. Typically, C ♀ × W ♂ and W ♀ × C ♂ matings produce larvae trisomic for chromosome 5, which frequently develop to the pupal stage before dying (Foster et al. 1976, and unpublished data), plus triploids which survive to adulthood (Maddern 1981; Foster and Whitten, unpublished, and see below). It is virtually certain that the other types of aneuploid zygotes produced by hybrid matings (i.e. monosomic for either the left or right or both arms of chromosome 5) are incapable of hatching. Broods which yielded viable adult offspring (G1 offspring) were progeny tested as follows. Broods which produced one or more homozygous *bp* offspring were noted. Male progeny of each brood (whether or not the *bp* phenotype was present) were mated both to compound *C(5L)3,to; C(5R)5,sby* and to chromosomally normal b^2/b^2*;w/w* females and the phenotypes of the offspring of these crosses (generally reared as a single culture) were recorded. If this test produced at least one *to* sby^+ or to^+*sby* offspring, the result was scored as a C × C mating. If the test produced wild-type ♀ and b^2 ♂ offspring, the result was scored as a W × W mating. Those tests which failed to produce any adult offspring, but in which the presence of *bp* in the G1 had been noted, were also scored as C × C matings. Tests which produced no adult or *bp* pupal offspring (i.e. "incomplete" tests) were treated as described in "Results".

Stage of ovarian development and mated status of trapped females

During November–February of the first season of the trial a portion of the females trapped was dissected for determination of stage of ovarian development (Vogt et al. 1974) and mated status.

Larvae from infested sheep

Larvae were obtained from infested sheep in the release area on two occasions. Larvae collected in April 1980 were reared on sheep liver in the laboratory to the late third-instar stage. Cytological examination of their mitotic chromosomes in air dried ganglion preparations (Bedo 1980) was then made. Larvae collected in April 1981 were reared to adulthood in the laboratory. Individual males were then test-mated to both b^2/b^2*; w/w* and *C(5L)3,to; C(5R)5,sby* females.

Results

The numbers of flies trapped during the 1979–80 season and data on population age-structure are listed in Table 5. Initially, flies trapped in the release area were few in number and comprised mainly mature

females. The large increase in fly numbers trapped on December 3 coincided with a dramatic shift towards a younger age structure. The numbers of flies trapped declined from mid-December 1979 to the end of February 1980, despite sustained releases of large numbers of larvae (Table 3), but increased again during March and April.

The results of progeny tests of inseminated females trapped during the 1979–80 season are presented in Table 6. No progeny tests were conducted on flies trapped prior to December 3, 1979. From then onwards, C×C matings were detected in all but one of the trappings made during the 1979–80 season. For the first three weeks of December, a majority of those progeny tests which produced viable offspring (i.e. non-hybrids) proved to represent C×C matings, with only a few W×W matings. For the next three weeks the W×W matings were more numerous than C×C matings. After mid-January, however, very few W×W matings were detected. Only 2 W×W compared to 200 C×C matings were detected in the last two months of the season. Presumptive hybrid matings represented a substantial portion of the total until the end of February, when their frequency declined markedly.

Also listed in Table 6 are the incomplete tests, i.e. those in which the trapped females produced viable adult offspring which themselves failed to produce offspring when mated to *b²; w* and *C(5L)3; C(5R)5*. Failure of these crosses to produce offspring was probably due to one of several causes, including failure to mate, sterility unrelated to the karyotypic status of the cross, or sterility of triploids produced by hybrid matings. These tests may be apportioned as outlined below.

In a series of crosses conducted with strains recolonized during the trial, 19 of 54 fertile (at least one larva hatched) egg masses from compound ♀×wild ♂ matings produced adult offspring (brood size range 1–31), nearly all of which were triploid males. In reciprocal crosses, one of 51 fertile cultures produced a single adult male (Mahon and Foster, unpublished). Triploid males produce no viable adult offspring when mated to either wild-type or compound females (unpublished data). The egg hatch distribution of the incomplete tests (Table 4B) suggests that most represented C×C matings. However, a 2×6 contingency table test between the egg-hatch distributions of the C×C and incomplete tests indicates significant heterogeneity ($X^2_{(5)} = 13.83$, $P < 0.05$), suggesting that a portion probably involved triploid offspring of hybrid matings. Empirical analysis of the egg hatch data for the completed tests (Table 4B) suggests a means of apportioning the incomplete tests, although such tests cannot fairly be used in statistical analysis. Of the 46 completed tests with 1–4% egg hatch, 39 (85%) were scored as hybrids and the rest as C×C matings, while of the 382 tests with 5–50% hatch, 323 were scored as C×C, 3 as W×W, (total 85%) and the rest as hybrids. If these findings are applied to the egg hatch data for the 94 incomplete tests, it can be estimated that 19 were probably hybrid matings, 74 C×C, and 1 W×W.

Analysis of the monthly progeny-test totals (Table 6) revealed a significant deficit of hybrid matings during December, but not from January onward. This was true whether the incomplete progeny tests were ignored, or were all assumed to represent C×C matings.

On April 21, 1980, 15 and 4 larvae respectively were sampled from two infested animals discovered in a flock of approximately 150 sheep. Examination of the chromosomes revealed that all 19 larvae taken were compound-chromosome bearing individuals.

The numbers of flies trapped during the 1980–81 season and the results of progeny tests of inseminated females, are listed in Table 7. The progeny-test results suggest that the majority of inseminated females trapped

Table 5. Trap catch and population age-structure data 1979–80

Date			Stage of ovarian development (No. of flies)					
	Number trapped		Nulliparous					Parous
	♀♀	♂♂	0	1	2	3,4	5	1–5
1979								
Nov. 5	15	0	–	–	–	–	–	–
19[a]	30	0	0	0	1	0	6	12
26[a]	14	0	0	2	2	1	1	4
Dec. 3	474	198	1	12	18	7	3	9
10	633	365	2	10	10	10	8	10
20	281	92	2	4	5	3	23	13
31[a]	150	53	2	9	7	1	3	10
1980								
Jan. 10	60	33	1	1	2	0	9	5
17	73	75	–	–	–	–	–	–
24	52	46	–	–	–	–	–	–
Feb. 6	124	67	1	9	6	2	3	1
13	58	36	–	–	–	–	–	–
20	61	20	1	2	3	1	2	1
25	2	2	–	–	–	–	–	–
Mar. 3	94	69	–	–	–	–	–	–
20[a]	241	248	–	–	–	–	–	–
26	412	280	–	–	–	–	–	–
Apr. 2	530	238	–	–	–	–	–	–
10	339	85	–	–	–	–	–	–
16[b]	> 196	> 18	–	–	–	–	–	–
22	284	89	–	–	–	–	–	–

[a] 10 traps only; otherwise 11 traps were used

[b] Not all flies trapped on April 16 were counted; flies were trapped April 30 and May 15–16, but were counted (see Table 6 for progeny-test results)

Table 6. Progeny tests of trapped inseminated females, 1979–80

Trapping date	No. of tests	Proportion of each mating type				Observed and expected[b] monthly totals (no. of tests)				$X^2_{(1)}$ OBS v EXP	
		Wild × Wild	Compound × Compound	Incomplete tests[a]	Hybrids	Wild × Wild	Compound × Compound	Incomplete	Hybrids	Completed tests[b]	All test[c]
Dec. 3, 1979	49	0.10	0.43	0.16	0.31	(OBS) 21(22)[d]	73 (93)	25	37 (41)		
10	45	0.09	0.51	0.20	0.20					14.18***	23.03***
20	42	0.07	0.57	0.12	0.24	(EXP) 11.9	63.8	–	55.0		
31	20	0.45	0.25	0.15	0.15						
Jan 10, 1980	25	0.16	0.24	0.04	0.56	(OBS) 6 (6)	20 (24)	5	20 (21)		
17	9	0.2	0.1	0.4	0.2					0.08	0.42
23	6	0	0.8	0	0.2	(EXP) 5.5	19.6	–	20.8		
31	11	0	0.7	0	0.3						
Feb. 6	28	0.14	0.32	0.11	0.43	(OBS) 5 (5)	22 (32)	15	19 (24)		
13	15	0.07	0.47	0.27	0.20					0.01	1.17
20	17	0	0.35	0.47	0.18	(EXP) 4.6	21.6	–	19.9		
25	1	0	0	0	1						
Mar. 3	18	0.11	0.78	0.06	0.06	(OBS) 2 (2)	69 (81)	15	4 (7)		
20	25	0	0.60	0.32	0.08					1.81	1.87
26	47	0	0.85	0.13	0.02	(EXP) 0.2	67.3	–	7.5		
Apr. 2	43	0	0.81	0.19	0						
10	35	0	0.74	0.20	0.06	(OBS) 2 (2)	146 (174)	34	14 (20)		
15	38	0	0.71	0.16	0.13					0.53	0.65
22	39	0.03	0.67	0.21	0.10	(EXP) 0.5	144.4	–	16.8		
30	30	0.03	0.77	0.13	0.07						
May 15, 16	12	0	0.75	0.08	0.17						

[a] See text

[b] Ignoring incomplete tests, expected totals were calculated as follows: $W \times W = (pW)^2$, $C \times C = (pC)^2$, $HYB = 2\ (pW.pC)$, where pW = proportion (Wild × Wild + 1/2 hybrid matings), pC = proportion (Compound × Compound + 1/2 hybrid matings)

[c] Assuming all incomplete tests are C × C, and re-calculating expected totals as above

[d] Figures in parentheses give total derived by apportioning incomplete tests as described in text

*** $P < 0.001$

during the first two weeks of the 1980–81 season, were CC females mated by CC males. This proportion declined during the next two weeks, and only 2 C × C matings compared to 30 W × W matings were detected in the November 25–December 16 period. More C × C matings were detected during January, but from January 29 onward no C × C matings were detected (compared to 54 W × W). In contrast to the data from January to May of the previous season, a significant deficit in the frequency of hybrid matings was evident for October and November, and January 13–21, when both W × W and C × C mating types were detected ($X^2_{(1)} = 11.06$, 32.16 and 11.80, respectively; $P < 0.001$ in each case). The distribution of egg hatches in the incomplete tests during October and November (Table 4 C) suggests that these were not hybrids. The presumptive hybrid mating detected in the April 21–22 trapping may have been an artefact caused by the use, starting in December 1980, of black filter papers (Schleicher and Schuell No. 551) for egg hatch counts. These were later found to be toxic to both eggs and larvae.

In April 1981 larvae were obtained from infested sheep on the same property as a year earlier. Sevently *L. cuprina* males reared from these larvae were individually progeny-tested. All proved to be wild males.

Discussion

The large increase in trap catch, coupled with the reduction in the average age of the population observed December 3, 1979, almost certainly reflected the emergence of the first flies released as larvae on November 14. The data on age and mated status (Table 5) indicate that released CC females were able to obtain the protein meal necessary to proceed beyond ovarian stage 1 (Vogt et al. 1974) and become inseminated. The recovery of CC larvae from sheep indicates that at least some CC females were capable of locating suitable oviposition sites in the field, and that the larvae were capable of development on sheep.

The progressive decline in fly numbers observed from mid-December until the end of February 1980, presumably resulted mainly from decreased survival in

Table 7. Trap-catch and female progeny-test data 1980–81

Trapping date	No. trapped		No. of tests	Proportion of each mating type			
	♀♀	♂♂		Wild × Wild	Compound × Compound	Incomplete tests	Hybrids
Oct. 22, 1980	21	5	9	0	1.0	0	0
27	35	4	14	0.07	0.79	0.14	0
28–30	45	17	26	0.15	0.69	0.15	0
Nov. 3	37	18	8	0	0.8	0.1	0.1
11	14	1	7	0.6	0.3	0.1	0
18	23	0	13	0.3	0.7	0	0
25	14	2	11	0.8	0.1	0.1	0
Dec. 11	19	4	8	1.0	0	0	0
16	40	29	16	0.81	0.06	0.13	0
Jan. 13, 1981[a]	20	0	12	0.8	0.2	0	0
21[b]	68	42	13	0.6	0.3	0.1	0
29[a]	29	4	26	0.85	0	0.15	0
Feb. 17	1	0	0	–	–	–	–
26	0	2	0	–	–	–	–
Mar. 5	2	1	0	–	–	–	–
Apr. 8	8	3	7	1.0	0	0	0
14–15	18	5	7	1.0	0	0	0
21–22	29	5	19	0.95	0	0	0.05
Apr. 28–May 1	0	0	0	–	–	–	–

[a] 10 traps only
[b] 8 traps only
[c] See text

the soil of the immature stages of both released and field-reared insects, due to warm summer soil temperatures (Wardhaugh et al. 1983; Dallwitz 1984). The increase in the number of flies trapped during March and April 1980 probably reflected a combination of the following: increased survival of the released larvae due to cooler soil temperatures, increased attractiveness of the traps due to the use of older baits (Woodburn and Vogt 1982), and an increase in the average weekly number of larvae released during March.

Both the size of the increase in trap catch on December 3, 1979 and the results of the progeny tests (Table 6), indicate that the flies resulting from released larvae greatly outnumbered those in the native population. The sizeable proportion of presumptive hybrid matings suggests that matings in the field between CC and wild flies were common. Because the released insects were not marked and cytological or other means of identifying females are not available, differentiation between the direction of matings, either C×W or W×C, of presumptive hybrid matings, is not possible. However, extensive data on segregation of compound chromosome arms in both male and female *L. cuprina* (Maddern and Foster, in preparation) suggest that C♀×W♂ matings should frequently yield higher hatch rates as compared with the reciprocal cross. Sample data from laboratory C×W and W×C crosses (Table 4A) support this. More than half of the presumptive hybrid matings had egg hatch values greater than 5% (Table 4B), suggesting that at least half represented W♀×C♂ matings. This, plus the progeny test data in Table 6, suggest that more than half of the wild females in the release area were mated by CC males during the first season of the trial.

Although W×W matings were detected only occasionally during the latter part of the 1979–80 season, the incidence of presumptive hybrid matings, plus the total trap catches, suggest the presence, at levels similar to those existing before December 3, of wild *L. cuprina* in the release area throughout this period. Thus despite the high CC : wild ratios suggested by the data, the wild population apparently was able to persist. This could reflect poor competitiveness of released males, or possibly immigration of wild flies. McKenzie (1976) found that immigration of wild *Drosophila melanogaster* was the main reason for the failure of a CC strain of this species to survive in a vineyard cellar, and he has shown (1977) that immigration rates as low as 0.5% inseminated females per generation can eliminate a CC population. Because of the relative isolation of the release area from other grazing areas, immigration seems unlikely to be the cause of elimination of the

Table 8. Distribution of progeny-test results by trap, October 1980

Date[a]	Trap no.																					
	1		2		3		4		5		6		7		8		9		10		11	
	C[b]	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W
30 Oct.					3	0			1	0	5	0	2	0	6	1	0	1	0	2	1	0
3 Nov.					1	0	2	0			1	0			2	0						
11 Nov.	0	1	0	2	1	0							1	0			0	1				
18 Nov.					0	1	5	0	0	1	2	2	1	0							1	0
Totals	0	1	0	2	5	1	7	0	1	1	8	2	4	0	8	1	0	2	0	2	2	0

[a] Trap number not recorded for flies trapped 22, 27 October
[b] C = C × C mating; W = W × W mating

L. cuprina CC strain in the present trial. It cannot be discounted entirely, however, since in separate studies Wardhaugh et al. (1983) detected marked *L. cuprina* 17 km downstream along a water course from the release point, and T. L. Woodburn (unpublished) has established that *L. cuprina* can occur in large forested tracts of rugged terrain.

The recovery of C × C matings during the 1980–81 season (Table 7) demonstrates the ability of the CC strain to overwinter in the field. Although the evidence of breeding of CC larvae on sheep in April 1980, suggests that some field-reared larvae could have overwintered, the vast majority of the C × C matings detected in October–November probably represented flies developed from the 12 million larvae released during April and May (Table 3). Dallwitz and Wardhaugh (1984) have shown that in the Canberra region, virtually all larvae entering the soil after the beginning of April, do not emerge until the following spring (i.e. late October–early November). The recovery of C × C matings as late as January 1981 suggests very strongly that the CC flies bred successfully in the field for at least one generation following the cessation of releases. Meteorological data (unpublished), coupled with data on developmental rates (Woodburn et al. 1978; Dallwitz 1984) suggest that at least one and at most two complete generations of breeding could have occurred at Brindabella between late October 1980 and mid-January 1981. However, the data from trapped flies and from larvae collected during April suggest strongly that the CC strain did not survive in the field after January 1981.

The data suggest that the deficit of hybrid matings observed during the first month of the trial (Table 6) was probably due to both a lag effect and an unusually low ♂ : ♀ ratio in the wild population. Firstly, the age-distribution of the wild population present in late November (Table 5) suggests that at least 90% of the wild females present when the CC flies first appeared, would have already been inseminated by wild males (L. Barton Browne, unpublished). Secondly, no males compared to 59 females were trapped during November, in contrast to the usual sex ratio of 1 : 2–3 in traps (e.g. Gilmour et al. 1946; Foster et al. 1978; Smith et al. 1981). Thus relatively few of the wild females would have been available for mating by CC males, and there were very few wild males to mate with the available CC females. The data for the rest of the first season (Table 6) contain no suggestion of assortative mating between the CC and wild strains.

Unlike the 1979–80 season, very few presumptive hybrid matings were detected during the 1980–81 season. Although there are insufficient data for statistical analysis of the causes, there is some suggestion that this apparent assortative mating may have been due to separation of the two strains in both time and space. The data in Table 7 suggest that CC flies were present somewhat earlier than the wild flies during October. Moreover, during the period early in the season when both W × W and C × C matings were detected, the two sorts of matings tended to be recovered from different traps (Table 8), suggesting that early in the season spatial separation may also have reduced inter-strain matings.

The deficiency of hybrid matings in the second season suggests that the failure of the CC strain to persist may have reflected lower fitness of the CC strain, rather than displacement by the wild strain through the mechanism of negative heterosis (which would have required more than the observed level of mating between the two strains). Conversely, the wild strain may have escaped elimination through this mechanism, merely by fortuitous temporal and spatial isolation from the CC strain, since the CC strain apparently was present initially during 1980–81 in sufficient numbers to approach, if not exceed, the theoretical

unstable equilibrium frequency (probably 80–90%), above which the CC strain should have replaced the wild strain. Observations made during mass rearing of the CC strain tended to corroborate the idea that this strain was less fit then non-CC strains. For example, females required 12–24 h more after eclosion to become gravid than translocation or wild-type strains. The likelihood that low fitness in CC strains can be attributed mainly to two types of inbreeding, has been discussed previously (Foster 1982).

Although the final result was the survival of the wild population and the disappearance of the CC strain, the demonstrated ability of released CC larvae to complete development, reproduce and overwinter in the field must be regarded as encouraging results in terms of the development of this genetic control strategy for *L. cuprina*.

New CC strains have been established by pooling offspring of trapped females which proved to represent C×C matings. These strains, which had in effect been selected for the ability to survive and mate (and in one case to overwinter) in the field, have recently been assessed in a further series of field and laboratory studies, the results of which will be reported elsewhere.

Acknowledgements. We thank Dr. D. G. Bedo for performing the cytological analyses, and Ms. V. Unsworth and Messrs K. G. Fenley and G. W. Crompton for competent technical assistance. Special thanks also to Dr. Tim Prout for his constructive comments on the manuscript.

References

Bedo DG (1980) C, Q and H-banding in the analysis of Y chromosome rearrangements in *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Chromosoma 77:299–308

Dallwitz R (1984) The influence of constant and fluctuating temperatures on development rate and survival in pupae of the Australian sheep blowfly (*Lucilia cuprina*). Entomol Exp Appl 36:89–95

Dallwitz R, Wardhaugh KG (1984) Overwintering of prepupae of *Lucilia cuprina* (Diptera: Calliphoridae) in the Canberra region. J Aust Entomol Soc 23:307–312

Fitz-Earle M, Holm DG, Suzuki DT (1973) Genetic control of insect populations: cage studies of chromosome replacement by compound autosomes in *Drosophila melanogaster.* Genetics 74:461–475

Foster GG (1980) Genetic control of sheep blowfly (*Lucilia cuprina*) and the logistics of the CSIRO control programme. Wool Technol Sheep Breed 28:5–10

Foster GG (1982) The use of bridging systems to increase genetic variability in compound chromosome strains for genetic control of *Lucilia cuprina* (Wiedemann). Theor Appl Genet 63:295–305

Foster GG, Whitten MJ, Prout T, Gill R (1972) Chromosome rearrangements for the control of insect pests. Science 176: 875–880

Foster GG, Whitten MJ, Konowalow C (1976) The synthesis of compound autosomes in the Australian sheep blowfly *Lucilia cuprina.* Can J Genet Cytol 18:169–177

Foster GG, Whitten MJ, Vogt WG, Woodburn TL, Arnold JT (1978) Larval release method for genetic control of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Bull Entomol Res 68:75–83

Foster GG, Maddern RH, Mills AT (1980a) Genetic instability in mass-rearing colonies of a sex-linked translocation strain of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) during a field trial of genetic control. Theor Appl Genet 58:169–175

Foster GG, Whitten MJ, Konovalov C, Bedo DG, Maddern RH, Boon DJ (1980b) Cytogenetic studies of *Lucilia cuprina dorsalis* (Wiedemann) (Diptera: Calliphoridae). Polytene chromosome maps of the autosomes and cytogenetic localization of visible genetic markers. Chromosoma 81:151–168

Foster GG, Whitten MJ, Konovalov C, Arnold JTA, Maffi G (1981) Autosomal genetic maps of the Australian sheep blowfly, *Lucilia cuprina dorsalis* R.-D. (Diptera: Calliphoridae), and possible correlations with the linkage maps of *Musca domestica* L. and *Drosophila melanogaster* (Mg.). Genet Res 37:55–69

Gilmour D, Waterhouse DF, McIntyre GA (1946) An account of experiments undertaken to determine the natural population density of the sheep blowfly, *Lucilia cuprina* Wied. Bull Coun Sci Ind Res, Melb No. 195

McKenzie JA (1976) The release of a compound-chromosome stock in a vineyard cellar population of *Drosophila melanogaster.* Genetics 82:685–695

McKenzie JA (1977) The effect of immigration on genetic control. A laboratory study with wild and compound chromosome stocks of *Drosophila melanogaster.* Theor Appl Genet 49:79–83

Maddern RH (1981) Radiation induced sex chromosome loss as an indicator of the optimal stage during spermatogenesis for the induction of compound chromosomes in *Lucilia cuprina.* Can J Genet Cytol 23:101–109

Smith PH, Konovalov CA, Foster GG, Kerr RW (1981) Assessment of the quality of mass-reared *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) males treated with dieldrin as larvae in a female-killing procedure. Bull Entomol Res 71:1–10

Steel RGD, Torrie JH (1960) Principles and procedure of statistics. McGraw-Hill, New York

Vogt WG, Havenstein DE (1974) A standardized bait trap for blowfly studies. J Aust Entomol Soc 13:249–253

Vogt WG, Woodburn JL, Tyndale-Biscoe M (1974) A method of age determination in *Lucilia cuprina* (Wied.) (Diptera: Calliphoridae) using cyclic changes in the female reproductive system. Bull Entomol Res 64:365–370

Wardhaugh KG, Smith PH, Crompton GW (1983) A comparison of ground and aerial release methods for the genetic control of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Genet Appl Entomol 15:37–46

Whitten MJ, Foster GG, Vogt WG, Kitching RL, Woodburn TL, Konovalov C (1977) Current status of genetic control of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). In: Proc 15th Int Cong Entomol. Washington DC, pp 129–139

Woodburn TL, Vogt WG (1982) Attractiveness of merino sheep before and after death to adults of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). J Aust Entomol Soc 21:131–134

Woodburn TL, Vogt WG, Kitching RL (1978) Estimation of age of females in field populations of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) using ambient temperature and solar radiation. Bull Entomol Res 68:251–261