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Identification of a male determinant on the X chromosome of housefly (*Musca domestica* L.) populations in South-East England

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SUMMARY

Houseflies collected from eight pig-breeding farms were used to investigate the nature of sex determinants in fly populations of South-East England. Earlier observations had shown that their sex determination mechanism was not of the standard (XX females, XY males) type.

Most flies of both sexes were XX; the male determining Y chromosome of standard populations was rare. Test-crosses to females of standard multimarked strains and crosses using aneuploid (OX) flies identified two dominant male determinants, one on autosome 3 (*M* III) and another on the X chromosome (X^m), and provided the first demonstration in this species of an active involvement of the X chromosome in sex determination. A small secondary constriction on X appeared to indicate reliably the presence of X^m. Most individuals in field populations were X^m homozygotes, implying the presence of an unlocated female determinant F,† epistatic to X^m and *M* III.

M III was less common and differed in frequency between samples. Its increased frequency in a strain selected in the laboratory with the pyrethroid insecticide permethrin might be due either to genetic drift, or to linkage between *M* III and a gene on autosome 3 that confers resistance to pyrethroids in houseflies.

1. INTRODUCTION

Houseflies have a perplexing variety of mechanisms of sex determination. Two types of population or strain are distinguished on the basis of the mechanism present: 'standard' (XX ♀♀, XY ♂♂) populations; and 'autosomal' populations with sex determinants on one or more of the five pairs of autosomes (XX ♀♀ and ♂♂).

In Europe the type and frequency of sex determinants present varies along a latitudinal cline (Franco, Rubini & Vecchi, 1982). Populations in Northern Europe are standard whereas those in Southern and Central Italy are autosomal and possess male determinants (*M* factors) on autosomes 2 and 3 (*M* II and *M* III) and a female determinant F, epistatic to *M*, on an undetermined autosome. 'Mixed'

† F is not italicized because its nature and location have not yet been resolved.

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populations in Northern Italy and at higher altitudes further south have Y chromosomes, *M* and F factors in varying proportions.

In common with populations in Denmark, Iceland, Holland and Germany (Franco *et al.* 1982), British houseflies would be expected to be predominantly of the standard type, but observations during genetic work on insecticide resistance in houseflies on pig-farms in South-East England showed that this is not so. This paper describes results of an investigation into the nature of the sex determination mechanism of these populations.

2. MATERIALS AND METHODS

(i) *Housefly strains*

(*a*) *Field strains*

The eight field strains collected between December 1980 and July 1981 from different pig-breeding farms within 15 km of Harpenden (35 km N.W. of London) are identified by code numbers of the farms (Fm3, Fm6, Fm9, Fm11, Fm13, Fm14, Fm22, Fm29). These farms supported housefly populations either throughout the year (nos. 3, 6, 11, 14, 22 and 29) or only in summer (nos. 9 and 13). A total of 50–150 adult flies collected from farrowing or weaner houses were reared in the laboratory for at least one generation before testing. The strains varied greatly in resistance to many insecticides (Sawicki *et al.* 1981, and unpublished data).

(*b*) *Laboratory strains*

Cooper, SRS (WHO Standard Reference Strain) – 2 wild-type strains, susceptible to insecticides, with a long history of laboratory culture. *ac*; *ar*; *bwb*; *ocra* – marked with recessive visible mutations on autosomes 1 (ali-curvae, *ac*), 2 (aristapedia, *ar*), 3 (brown-body, *bwb*) and 5 (ocra-eyes, *ocra*); insecticide susceptible. *ac*; *ar*; *bwb*; *ye* – marked with recessive visible mutations on autosomes 1, 2, 3 (as above) and 4 (yellow-eyes, *ye*); insecticide susceptible.

All four laboratory strains have the standard XY sex determination mechanism.

(ii) *Rearing methods*

Mass crosses involved at least 50 flies of each sex. Single-pair crosses were set up in plastic cups with gauze-covered bottoms through which females oviposited on to cotton-wool rolls soaked in milk. Adults were fed on water, sugar and fresh milk, larvae on a bran-based medium containing dried milk and yeast powder. Virgin females were collected from rearing cages within 18 h of emergence.

(iii) *Cytogenetic studies*

Karyotypes were examined, usually at the first generation of laboratory culture, in squashes of gonads stained with acetolactic-orcein (cf. Rubini, Vecchi & Franco, 1980).

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(iv) *Genetic analyses*

Mass crosses between field and standard strains provided preliminary information on the nature and frequency of sex determinants present in field strains. Single-pair crosses between field strain males and standard females disclosed the frequency of males responsible for a sex-ratio departing from 1:1 in F_1 progeny.

To determine the linkage relationships of male determinants in two field strains (Fm6 and Fm22), F_1 (multimarked ♀ × field ♂) males were test-crossed with multimarked females in single-pairs, and progeny were scored for sex and phenotype as described below.

3. THEORETICAL CONSIDERATIONS

This section details the expected segregations and sex-ratios from crossing flies with different sex-determining mechanisms. The sex determinants discussed are: (i) Y chromosome acting as male determinant (standard mechanism); (ii) male and female determinants on autosomes; and (iii) male determinant on X.

(i) In standard strains X is inert for sex determination and only the smaller Y acts as a dominant male determinant (Milani, 1967; Hiroyoshi, 1977). The sex-ratio in progenies of crosses between standard strains is normal (1 ♀:1 ♂).

(ii) Autosomal strains lack Y and always have male determinants (*M*) and generally female determinants (F) on the autosomes. Dominant *M* factors occur on autosomes 1, 2, 3 and 5 in populations of diverse geographic origin (for references see Franco, Rubini & Vecchi, 1982). F factor(s) epistatic to *M* factors have only been found on autosome 4 (McDonald *et al.* 1978; Inoue & Hiroyoshi, 1982).

Sex-ratios expected from single-pair crosses between standard XX females and males homozygous or heterozygous for 1 or 2 unlinked *M* factors are:

	Genotype of male parent	Sex-ratio
Cross 1	$M_1/+$	1 ♀:1 ♂
Cross 2	$M_1/+\,;\, M_2/+$	1 ♀:3 ♂
Cross 3	M_1/M_1	All male

Homozygosity for one *M* factor in cross-3 masks the influence of other *M*'s present. Sex-ratios from mass crosses of standard females and *M*-bearing males vary according to the number, frequency and extent of homozygosity for *M* factor(s) in the field population.

The linkage of *M* factors is established from single-pair test-crosses to standard XX females marked with visible recessive mutants on all the autosomes thus:

ac; *ar*; *bwb*; *ocra* ♀ × F_1(*ac*; *ar*; *bwb*; *ocra* ♀ × *M* ♂) ♂
ac; *ar*; *bwb*; *ye* ♀ × F_1(*ac*; *ar*; *bwb*; *ye* ♀ × *M* ♂) ♂

Since recombination in males is rare or absent (cf. Rubini, Vecchi & Franco,

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1980) the expected segregations and sex-ratios with two factors, M_1 and M_2, segregating from recessive markers r_1 and r_2 respectively are:

Scheme 1. Only M_1 present

$$\frac{+r_1}{+r_1}\,♀ \quad \times \quad \frac{+r_1}{M_1+}\,♂$$

$$\downarrow$$

$$\frac{+r_1}{+r_1}\,♀ \quad : \quad \frac{+r_1}{M_1+}\,♂$$

Expected sex-ratio 1 ♀:1 ♂; all males wild type and all females r_1.

Scheme 2. M_1 and M_2 present

$$\frac{+r_1}{+r_1};\frac{+r_2}{+r_2}\,♀ \quad \times \quad \frac{+r_1}{M_1+};\frac{+r_2}{M_2+}\,♂$$

$$\downarrow$$

$$\frac{+r_1}{+r_1};\frac{+r_2}{+r_2}\,♀ \quad : \quad \frac{+r_1}{+r_1};\frac{+r_2}{M_2+}\,♂ \quad : \quad \frac{+r_1}{M_1+};\frac{+r_2}{+r_2}\,♂ \quad : \quad \frac{+r_1}{M_1+};\frac{+r_2}{M_2+}\,♂$$

Expected sex-ratio 1 r_1, r_2 ♀:3(1 r_1, + : 1+, r_2: 1+, +) ♂.

Scheme 3. M_2 is not locatable when multimarker strain lacks r_2

$$\frac{+r_1}{+r_1};\frac{++}{++}\,♀ \quad \times \quad \frac{+r_1}{M_1+};\frac{++}{M_2+}\,♂$$

$$\downarrow$$

$$\frac{+r_1}{+r_1};\frac{++}{++}\,♀ \quad : \quad \frac{+r_1}{+r_1};\frac{++}{M_2+}\,♂ \quad : \quad \frac{+r_1}{M_1+};\frac{++}{++}\,♂ \quad : \quad \frac{+r_1}{M_1+};\frac{++}{M_2+}\,♂$$

Expected sex-ratio 1 r_1 ♀:3(1 r_1:2+) ♂.

To identify the linkage of M_2, males are test-crossed to females marked with r_2.

(iii) We report here for the first time on strains in which X bears an active dominant male determinant. Since nothing is known of the nature of this determinant, the symbol X^m is used here to denote both the X-linked male determinant and an X chromosome that bears it. X denotes a standard X chromosome lacking X^m. Expected sex-ratios from single-pair crosses between XX females and X^m-bearing males are:

	Genotype of male parent	Sex-ratio
Cross 4	X^m/X	1 ♀:1 ♂
Cross 5	X^m/X^m	All male

The sex-ratios from mass crosses vary according to the proportion of males homozygous for X^m.

Since X^m is not autosomal, test-crosses involving males with X^m only show no sex-limited expression of markers (Scheme 4):

Scheme 4. X^m only

$$\frac{X}{X};\frac{+r_1}{+r_1}\,♀ \quad \times \quad \frac{X^m}{X};\frac{++}{+r_1}\,♂$$

$$\downarrow$$

$$\frac{X}{X};\frac{+r_1}{+r_1}\,♀ \quad : \quad \frac{X}{X};\frac{+r_1}{++}\,♀ \quad : \quad \frac{X^m}{X};\frac{+r_1}{+r_1}\,♂ \quad : \quad \frac{X^m}{X};\frac{+r_1}{++}\,♂$$

Expected sex-ratio 2 ♀(1r_1 and 1+):2 ♂(1r_1 and 1+).

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X^m may also coexist with one or more M factor(s) (Scheme 5):

Scheme 5. X^m, $+M_1$ on homologous autosome to that bearing r_1

$$\frac{X}{X};\frac{+r_1}{+r_1}\,♀ \times \frac{X^m}{X};\frac{M_1+}{+r_1}\,♂$$

$$\downarrow$$

$$\frac{X}{X};\frac{+r_1}{+r_1}\,♀ : \frac{X^m}{X};\frac{+r_1}{+r_1}\,♂ : \frac{X}{X};\frac{+r_1}{M_1+}\,♂ : \frac{X^m}{X};\frac{+r_1}{M_1+}\,♂$$

Expected sex-ratio 1 r_1♀:3 ♂(1 r_1 and 2+).

In Scheme 5, unlike Scheme 3, the 2nd test-cross to females marked on the remaining autosomes shows X^m to be non-autosomal.

Aneuploid (OX) flies lacking one sex chromosome can provide direct evidence of the association between the male determinant X^m and the X chromosome. Crosses between standard XX females and OX (hypothesized OX^m) males yield only OX females and XX^m males (Scheme 6*a*). Crosses between OX females and XX (hypothesized XX^m) males yield both OX^m and XX^m males (Scheme 6*b*) which when crossed in single-pairs to OX females should produce families differing in sex-ratio (Scheme 6*b* and *c*):

Scheme 6. Crosses involving aneuploid andX^m flies:

(*a*) XX♀ × OX^m♂ → OX♀:XX^m♂ — 1♀:1♂

(*b*) OX♀ × OX^m♂ → OO:OX♀:XX^m♂:OX^m♂ (OO assumed lethal) — 1♀:2♂

(*c*) OX♀ × XX^m♂ → XX♀:OX♀:XX^m♂:OX — 1♀:1♂

Expected sex-ratio

Hence if X^m segregates with the X chromosome, single-pair progeny of crosses involving OX^m or XX^m males, identified by the karyotype of the male, should show a sex-ratio of 1 ♀:2 ♂ and 1 ♀:1 ♂ respectively.

4. RESULTS

(i) *Karyotypes of field strains*

In all field strains most males and females were XX (Table 1). Typical Y chromosomes were found in only four of the eight strains examined (Fm3, Fm14, Fm22, Fm29) and occurred in both sexes in three of them. Hence the sex-determination mechanism in these strains was largely independent of Y and thus non-standard. Aneuploidy of the X chromosome was uncommon.

(ii) *Crosses with standard strains*

Mass crosses between Cooper females and field strain males produced a large and consistent excess of males (83·6–95·6 % of total progeny), whereas the sex-ratio from reciprocal crosses was approximately normal (Table 2). All crosses also produced a variable but small proportion of gynandromorphs or intersexes (Milani, 1967) that could not be ascribed to either sex. Such flies were not examined further.

Pooled F_1 sex-ratios of single-pair crosses between males of four field strains (Fm6, Fm9, Fm13, Fm22) and SRS or *ac*; *ar*; *bwb*; *ocra* females were similar to

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those described above (Table 3*a*). Individual male parents contributed unequally to these aberrant sex-ratios since a small number of single-pair families produced by three field strains contained *c.* 50% females; most families consisted entirely of males or included a very small number of (usually < 2%) females. Hence most male parents were homozygous for a dominant male determinant, either autosomal or X^m (Cross 3 or 5, Section 3). Males fathering the few families with a normal sex-ratio were heterozygous for this or another factor (Cross 1 or 4).

Table 1. *Sex chromosome karyotypes observed in eight field strains*

	Females				Males				
Strain	No. examined	XX	XO	XY	No. examined	XXX	XX	XO	XY
Fm3	25	22	0	3	19	0	15	0	4
Fm9	33	33	0	0	33	0	33	0	0
Fm6	24	24	0	0	36	0	32	4	0
Fm13	22	22	0	0	29	0	29	0	0
Fm11	—	—	—	—	11	0	11	0	0
Fm14	26	24	1	1	27	0	24	0	3
Fm22	20	19	1	0	46	0	45	0	1
Fm29	25	17	3	5	22	1	18	0	3
Total	175	161	5	9	223	1	207	4	11
% XX flies		92·0					92·8		

Table 2. *Sex-ratio of F_1 progeny of mass-crosses between field and Cooper strains*

Field parent	No. scored	♀	♂	?*	Sex-ratio†
	F_1 (Field ♀ × Cooper ♂)				
Fm3	976	501	433	42	0·447
Fm6	657	374	273	10	0·416
Fm11	1030	500	524	6	0·508
Fm14	915	415	500	0	0·546
Fm22	958	458	500	0	0·522
Fm29	1021	502	519	0	0·508
	F_1 (Cooper ♀ × Field ♂)				
Fm3	569	37	500	32	0·879
Fm6	523	18	500	5	0·956
Fm11	547	1	500	46	0·914
Fm14	598	84	500	14	0·836
Fm22	529	58	500	14	0·945
Fm29	536	6	500	30	0·933

* ? = gynandromorphs or intersexes.
† No. ♂/total no. progeny, i.e. including intersexes.

(iii) *Linkage of male determinants*

Only *bwb* on autosome 3 showed evidence of sex-linked inheritance in test-cross progeny. Three types of family differing in phenotypic segregation of *bwb* and overall sex-ratio were distinguished (Table 4 shows an example of each).

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Type A: sex-ratio 1 ♀:1 ♂, segregation of *bwb* independent in both sexes.
Type B: sex-ratio 1 ♀:3 ♂, one-third of males and all females *bwb*.
Type C: Sex-ratio 1 ♀:1 ♂, all males wild type and all females *bwb*.

Family types A, B and C were expected from crosses detailed in Schemes 4, 5 and 1 respectively, indicating the presence in field strains of both X^m and an autosome 3 factor (*M*III). Single-pair crosses also showed that the proportion of family types

Table 3. *Sex-ratio of F_1 progeny of single-pair crosses between field strain males and standard females*

(*a*) Pooled data for all pairs

Origin of father	Origin of mother	F_1 progeny: No. scored	♀	♂	?*	Sex-ratio†
Fm9	SRS	1736	221	1490	25	0·858
Fm6(a)	SRS	4541	415	4115	11	0·906
Fm6(b)	*ac*: *ar*; *bwb*; *ocra*	3871	324	3544	3	0·916
Fm13	SRS	1131	2	1122	7	0·992
Fm22	SRS	4613	132	4334	147	0·940

(*b*) Analysis of progeny of individual pairs

Origin of father	Total no. of pairs	No. of pairs producing: < 2% females	2–12%	*ca.* 30%	*ca.* 50%
Fm9	16	9	3	1	3
Fm6(a)	38	31	0	0	7
Fm6(b)	65	55	0	0	10
Fm13	11	11	0	0	0
Fm22	35	33	0	0	2

*,† See footnote to Table 2.

Table 4. *Examples of* bwb *segregation and sex-ratio in test-cross progeny*

Pair no.	♀ +	♀ bwb	♀ Total	♂ +	♂ bwb	♂ Total	Family type*
3370	67	58	125	59	64	123	A
3427	0	23	23	44	16	60	B
3465	0	17	17	24	0	24	C

* See text for explanation.

in test-cross progeny, and hence the frequency of X^m and *M*III in parental males differed in Fm6 and Fm22 (Table 5). In both strains almost all families (97–100%) had X^m (Type A and B families combined), indicating that it was virtually homozygous in field strain males, but *M*III (Type B and C families combined) was much less common in Fm6 males. Type C families (*M*III only) were found only in the first experiment with Fm22 when the proportion of males with both X^m and *M*III (0·291) was close to that expected (0·31) by multiplying the proportions of X^m-bearing (0·971) and *M*III-bearing (0·32) males.

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The 4th test-cross (Fm22se1, Table 5) used Fm22 males selected for three successive generations with permethrin (to be published). These selections raised the LD50 to permethrin of this strain from 0·41 μg per fly to *c.* 12 μg per fly and increased the frequency of *M* III from *c.* 29 % pre-selection to 72 %, two generations after the 3rd selection.

Table 5. *Distribution of family types in test-cross progeny*

			Family type					
			A (X^m only)		B (X^m + *M* III)		C (*M* III only)	
Field strain	Standard strain	Total no. pairs	No.	%	No.	%	No.	%
Fm6	*ac*; *ar*; *bwb*; *ye*	48	45	93·8	3	6·2	0	—
Fm22	*ac*; *ar*; *bwb*; *ocra*	103	70	68·0	30	29·1	3	2·9
Fm22	*ac*; *ar*; *bwb*; *ye*	21	15	71·4	6	28·6	0	—
Fm22sel	*ac*; *ar*; *bwb*; *ocra*	125	35	28·0	90	72·0	0	—

(iv) *Crosses involving aneuploid flies*

F_1 progenies of two of the single-pair matings between XX females and field strain males that showed a normal sex-ratio (Table 3*b*) consisted entirely of OX females and XX (assumed XX^m) males, and were likely to have resulted from a cross between a standard female and an aneuploid OX^m male (Scheme 6*a*). Males produced by inbreeding these progeny should be XX^m and OX^m (Scheme 6*c*). Such males were crossed in single pairs to their OX aunts (Scheme 6*b* and *c*), and male parents were scored for karyotype once eggs had been laid. Single-pair progeny of males with one or two X chromosomes were stored separately, and their sex-ratios were recorded.

χ^2 tests indicated that progeny of OX^m and XX^m males conformed to predictions detailed in Scheme 6 (Table 6, series 1), and that results for individual families fathered by each type of male were homogeneous. Since the cross OX ♀ × OX^m ♂ produced only OX females but both XX^m and OX^m males (Scheme 6*b*), a 2nd series of single-pair crosses between these progeny should have and did replicate the 1st series (Table 6, series II). Pooled results for individual families of both series were as homogeneous as those within each series. The close agreement between predicted and observed results confirmed that X^m segregated with the X chromosome.

6. DISCUSSION AND CONCLUSIONS

The sex determination mechanism in housefly populations of South-East England involves at least three independent dominant male determinants (X^m, *M* III and Y). Most males in the field strains examined were homozygous for X^m, which segregates with X chromosomes. *M* III, on autosome 3, was present in about one-third of males of Fm22 but only 6 % of males of Fm6. The typical male determining Y chromosome of standard strains was rare and its effect was masked by *M* III and X^m.

The presence of X^m homozygotes implied the existence in field strains of a dominant female determinant, F, whose location was not investigated. However,

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Table 6. *Pooled sex-ratios of single-pair progeny of aneuploid flies*

Series of crosses	Karyotype of father	Expected sex-ratio	No. of pairs	No. of progeny ♀	No. of progeny ♂	χ^2 analysis $\Sigma\chi^2$	D.F.	χ^2s	D.F.	χ^2_h*	D.F.	$P\ \chi^2_h$
I	OX^m	1 ♀:2 ♂	4	57	144	4·08	4	2·24	1	1·84	3	0·61
II	OX^m	1 ♀:2 ♂	8	150	293	4·44	8	0·06	1	4·38	7	0·74
Both series			12	207	437	8·52	12	0·41	1	8·11	11	0·70
I	XX^m	1 ♀:1 ♂	6	202	193	3·75	6	0·20	1	3·55	5	0·62
II	XX^m	1 ♀:1 ♂	14	635	647	4·99	14	0·11	1	4·88	13	0·98
Both series			20	837	840	8·74	20	0·01	1	8·73	19	098

* Homogeneity $\chi^2_h = \Sigma\ \chi^2$ (individual families) $- \chi^2_s$ (families pooled).

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its presence in field strain females was confirmed by mating males of Fm6 twice, first to a standard female and then to a Fm6 female. Males fathering all-male progeny in the first cross invariably produced males and females in the second cross. In a Florida strain of houseflies, F was found to be a complex of numerous, closely linked genes that could split during meiosis to produce gynandromorphs or intersexes (Rubini, Franco & Vanossi Este, 1972). The appearance of such abnormal flies in some of the crosses described above (Tables 2 and 3) suggests that in British populations F may be similarly complex. The homozygosity of X^m in most F-bearing field strain females accounts for the sex-ratio of progeny of such females and standard XY males being close to 1 ♀:1 ♂ (Table 2) rather than 3 ♀:1 ♂ as expected if females possess F without *M* factors.

The sporadic appearance of females in otherwise all male progeny of single-pair crosses between standard females and field strain males (Table 3*b*) is, *a priori*, difficult to reconcile with the view that male parents were X^m homozygotes. However, all such females examined were OX whereas their male brothers were XX (i.e. XX^m) or exceptionally XXX. Such aneuploidy probably reflects meiotic non-disjunction in the X^mX^m father causing OX daughters to inherit only the maternal X (non X^m) chromosome.

Both X and Y chromosomes of field strains varied in appearance (Plate 1). Two forms of Y differing slightly in length and arm-length ratio were recognized (Plate 1*f*, *g*) while the larger and typically isobrachial X (Plate 1*a*) also occurred as a smaller (Plate 1*b*) and sometimes moderately heterobrachial (Plate 1*c*, *f*) variant. Similar variation has been documented in other studies and does not appear to influence sex determination (Rubini, 1967; Rubini *et al.* 1972). A third, previously unknown variant of X had a small secondary constriction in one arm that was conspicuous in early metaphase (Plate 1*d*) but apparently absent in late metaphase in the same individual when chromosomes are more contracted. Only one X chromosome of XX^m fathers of crosses reported in Table 6, and the sole X chromosome of OX^m fathers showed this constriction; hence this variant may be the X^m-bearing chromosome.

The evolution of non-standard sex determination mechanisms in houseflies appears to be a recent phenomenon. The frequency of *M* factors increased markedly in Japanese and Italian populations between 1960 and 1975 (Hiroyoshi, 1980; Franco *et al.* 1982). There are no comparable data for British populations, but we have evidence that X^m is less frequent in the north than in the south (to be published). The concomitant spread of autosomal sex determinants and insecticide resistance in housefly populations throughout the world prompted Hiroyoshi (1980) to suggest that the phenomena are causally related, although he no longer holds this view (pers. comm. to R. M. Sawicki, 1982). At present there is no substantive evidence that non-standard sex determinants play a role in resistance since reports of an association between *M* factors and resistance genes (Kerr, 1961, 1970; Milani, 1962; Rupes & Pinterova, 1975) can also be attributed to tight linkage or drift. This may also explain the increase in *M* III in the Fm22sel strain following selection with permethrin. Drift is likely to be an important determinant of gene frequencies in populations drastically reduced in size by insecticides (Krimbas & Tsakas, 1971).

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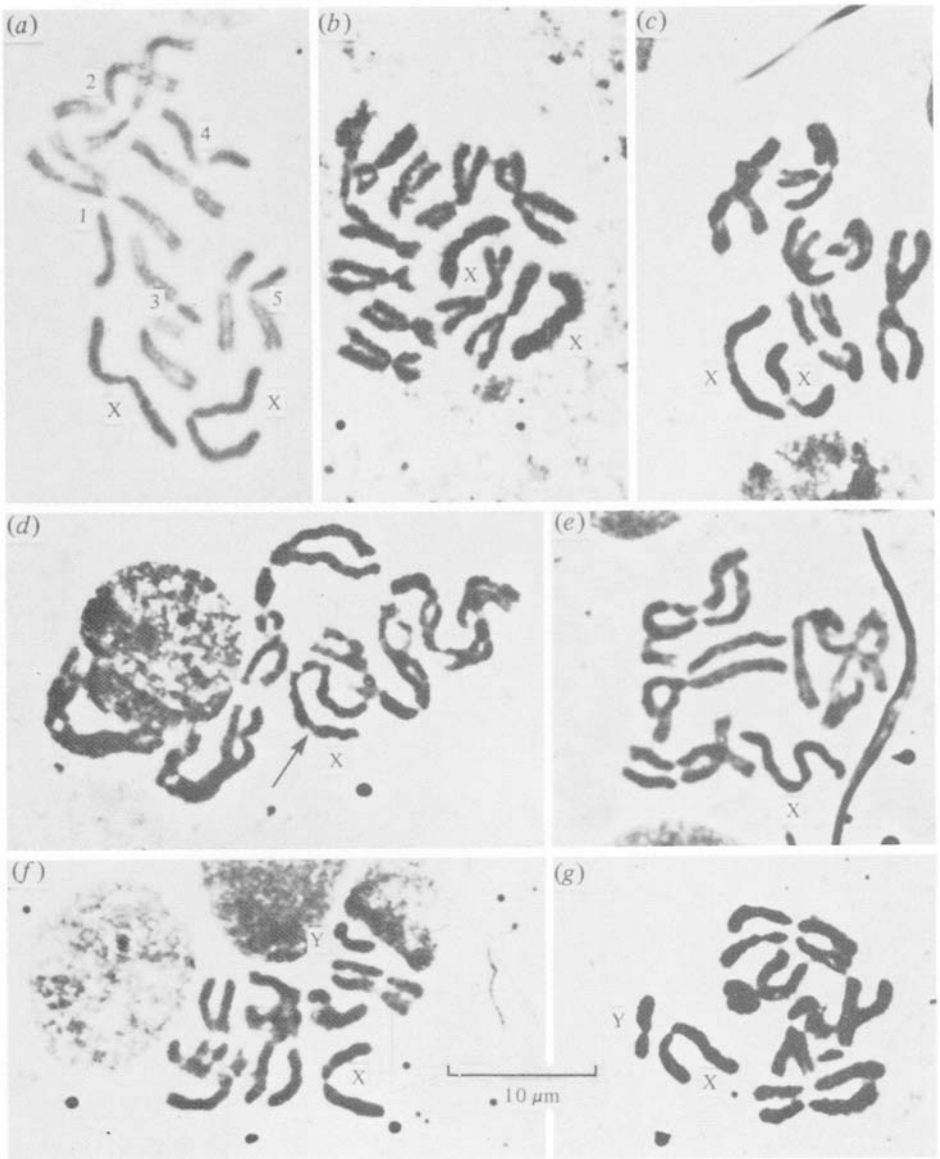

Variants of X and Y chromosomes observed in field populations of houseflies (photographed at mitotic metaphase from squashes of gonads). (*a*) XX ♂ from Fm22, X chromosomes similar; (*b*) and (*c*) XX ♂♂ from Fm3 (*b*) and Fm22 (*c*), X chromosomes of unequal length; (*d*) and (*e*) the same OX ♂ from Fm22, the X chromosome showing a clear secondary constriction (arrowed) in early metaphase (*d*) that is not apparent in late metaphase (*e*); (*f*) XY ♂ from Fm22, typical small Y; (*g*) XY ♂ from Fm3, X heterobrachial and Y larger than in (*f*).

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Third-autosome *M* factors have now been detected in field strains collected in Britain, Italy (Franco *et al.* 1982), North America (McDonald *et al.* 1975), Japan (Hiroyoshi & Fukumori, 1977, 1978; Tsukamoto, Sono & Horio, 1980) and Fiji (Hiroyoshi & Inoue, 1979). It should be stressed that although all these are by convention termed *M*III, their homology is unproven. *M*III probably originated independently in Britain because it is absent from other countries of Northern Europe although it is frequent in Italy.

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