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Sex-determinants and their distribution in various populations of *Musca domestica* L. of Western Europe

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SUMMARY

The distribution of sex-determinants in field populations of *Musca domestica domestica* L. was studied in 62 samples of flies collected at 53 sites (animal farms) between 1975 and 1981 in an area stretching North–South from Denmark (+Iceland) to Sicily.

Karyological observations and genetic analyses demonstrated the existence of three types of population along a latitudinal cline. Populations of Northern Europe were of the standard type (*XX* females and *XY* males) with the *Y* chromosome determining sex. Those of Central and Southern Italy from sites below 100 m.a.s.l. (metres above sea level) were autosomal (*XX* females and males), sex in them being determined by autosomal sex-determinants for both femaleness and maleness. In the large intermediate zone the populations were mixed and had several karyotypes in both sexes. In this zone an altitudinal gradient was also observed, with autosomal determinants less common at higher altitudes. Genetic tests showed, in the autosomal and in the mixed populations, the presence of two autosomal male factors: *M* III, the most common, on autosome III and *M* II, on autosome II.

The gradient in sex determinants found in flies of Western Europe appears to be a dynamic phenomenon of relatively recent origin. Both climatic influence and selective pressure with insecticides have probably contributed towards the micro-evolution of populations with different sex-determinants in the houseflies of the area studied.

1. INTRODUCTION

All the papers concerning the karyotype of *Musca domestica* L. ($2n = 12$) published between 1908 and 1948 (Stevens, 1908; Metz, 1916; Keuneke, 1924; Perije, 1948) and some published later (Ramade, 1961; Milani, 1964*a*; Rubini, 1967) reported the presence of *XX* females and *XY* males, these chromosomes being completely heterochromatic. It can be assumed that the authors, being European, examined houseflies of European origin.

Since 1958, cases of sex-limited inheritance, interpreted *a posteriori* as due to autosomal sex-determinants, have been described in several strains of houseflies of non-European origin (Sullivan, 1958; Milani & Franco, 1959*a*; Kerr, 1960, 1961;

Sullivan, 1961; Milani, 1962; Tsukamoto, Baba & Hiraga, 1961; Franco, Lanna & Milani, 1962; Hiroyoshi, 1964).

Cytological and genetic analyses of crosses between an European 'standard' strain (SRS: *XX* females, *XY* males) and an 'atypical' strain from Florida (Lab.-1: *XX* females and males) showed that Y chromosome is the sole sex-determinant in the standard strain (Milani, Rubini & Franco, 1967). In the atypical strain a male determinant *M* on autosome III, *M* III, was present in both sexes, and females had an autosomal female determinant F[1] epistatic to *M*. Viable and fertile males and females were isolated with all combinations of *X* and *Y* chromosomes, mostly disomic: *XX–XY–YY*, but sometimes aneuploid: *XO–XXX–OY–XXY–XYY–YYY*.

Subsequent studies (Rubini & Franco, 1968; Milani, 1971; Rubini, Franco & Vanossi Este, 1972) suggested that F is polygenic, and that *M* III, localized in relation to three visible markers, is probably near the centromere of autosome III (Rubini & Franco, 1972).

Since 1969, other autosomal sex-determinants have been described in houseflies of non-European origin as listed below:

M II, *M* III, *M* V, F, Australian laboratory strain (Wagoner, 1969);
M II, Australian DDT-resistant laboratory strains (Kerr, 1970);
M III, North American field populations (McDonald *et al.* 1975);
M III, F, Japanese field populations (Hiroyoshi and Fukumori, 1977, 1978);
F IV, compound laboratory strain (North American-Australian), (McDonald *et al.* 1978);
M I, *M* II, *M* III, F, Fijian field population (Hiroyoshi & Inoue, 1979);
M I, *M* II, *M* III, *M* V, Japanese field populations (Tsukamoto, Shono & Horio, 1980).

In Europe only two autosomal sex-determinants have been reported:
M III, Czeck DDT-resistant laboratory strains (Rupes & Pinterova, 1975);
F (probably polygenic), North Italian field population (Rubini, van Heemert & Franco, 1977).

This work amplifies the study of geographic distribution in houseflies of European origin, whose preliminary results were briefly reported elsewhere (Franco, Rubini & Vecchi, 1979; Rubini, Franco & Vecchi, 1980).

2. MATERIALS AND METHODS

All the samples of houeflies examined belonged to *Musca domestica domestica* L., the only subspecies present in Europe (Saccà, 1967).

[1] F is not italicized because it is not localized and its nature is not fully clarified.

(*i*) *Housefly strains*

(*a*) *Field populations*

62 samples of houseflies were collected between 1975 and 1981 at 53 sites between Denmark (+ Iceland) and Southern Italy.

(*b*) *Laboratory strains*

These were SRS/*Musca domestica*/1, a standard reference strain of wild phenotype (SRS in the following); the marked strains *ac; ar; bwb; ocra* (I, II, III; V) and *ye* (IV).

All the laboratory strains had the normal *XX* (♀♀) and *XY* (♂♂) sex-chromosomes and were without autosomal sexual determinants.

(*ii*) *Collecting and rearing*

Adult houseflies were collected with a sweep net on animal breeding farms. All the samples, except two collected in October, were caught during the Summer months and, except those specified, only one sample was collected at each site. The flies were bred in the laboratory, care being taken to avoid undue selective pressures.

(*iii*) *Karyological examinations*

Squashes of the gonads of both sexes of field collected flies (parents) and/or laboratory reared progenies (F_1) were examined karyologically after staining with aceto-lactic orcein, according to the techniques already described (Rubini & Palenzona, 1967; Rubini, Vecchi & Franco, 1980).

(*iv*) *Genetic analysis*

(*a*) *Sex-determinants in males*

Males of the field populations, mostly of F_1, were crossed in single pairs with virgin females of the SRS strain, as shown in Scheme 1. This, combined with karyological observations of the parents and sex-ratio controls of the progeny, should reveal the presence of sex-determinants in parental males.

To identify the linkage group(s) of factor(s) *M*, *XX* males, proven carriers of *M*, were usually crossed with females *ac; ar; bwb; ocra* and sometimes with females *ye* as follows: parental cross: ♀ *XX* marked × ♂ *XX* wild; test cross: ♀ *XX* marked × ♂ *XX* F_1 hybrid.

Because of the absence (Hiroyoshi, 1961; Tsukamoto, 1964) or rarity (Milani, 1956; Sullivan, 1961; Rubini, Vecchi & Franco, 1980) of crossing over in the male, recessive markers, are expected to segregate from the male sex-determinant when marker(s) and *M* belong to homologous autosomes. Thus, when marker *bwb* on autosome III and *M* III are involved, all females are *bwb/bwb* and all males are *bwb/bwb*$^+$ (Milani, Rubini & Franco, 1967).

Scheme 1. *Summary of the results which can be obtained from genetical tests of ♂♂ of* Musca domestica *L. by single pairs*

Cross: *XX* standard ♀ × ♂ of a field population

Karyotype observed in the tested ♂ (1)	Sex-ratio obtained in the F_1 (2)	Sex determinants* present in the tested ♂ (deduced from 1 and 2)
XY	1♀:1♂	*Y*
XY	1♀:3♂♂	*YM*
XY	1♀:7♂♂	YM^aM^b
XY	All ♂♂	*YMM*
XX	1♀:1♂	*M*
XX	1♀:3♂♂	M^aM^b
XX	All ♂♂	*MM*
YY	All ♂♂	*YY*

* The symbol *M* is used to indicate any autosomal male determining factor. The symbols M^aM^b are used to indicate two non-linked autosomal male determining factors. For simplicity, in any case the minimum number of *M*, necessary to explain the sex-ratio, is reported. For example, an *XX*♂, parent of an all-♂♂ progeny, might also be provided with $M^aM^aM^b$, $M^aM^bM^b$, $M^aM^aM^bM^b$.

(*b*) *Sex-determinants in females*

Females of field populations were usually examined karyologically. In each population the presence of F, epistatic to both *Y* and *M*(s) (Milani, Rubini & Franco, 1967; Rubini, van Heemert & Franco, 1977), was deduced from the presence of female *Y* carriers or males homozygous for *M* or *Y*, since both *MM* and *YY* males may be produced only by female carriers of male determinant(s) and therefore provided with F.

Where genetic tests (Scheme 1) showed no *M*, the presence of F was tested by the single pair mating of F_1 virgin females of field populations with SRS males. A 3 ♀♀:1 ♂ sex ratio in the progeny should reveal the presence of F in the parental female (Milani, Rubini & Franco, 1967).

3. RESULTS

(*i*) *Karyological observations and genetic analysis of sex-determinants in field populations*

Of 53 field populations examined, 12 (13 samples) called 'standard' always had a single *Y* chromosome in males, 11 called 'autosomal' totally lacked *Y* in both sexes, and 30 (38 samples) called 'mixed' had several karyotypes in both sexes.

(*a*) *Standard populations*

All 12 populations collected North of Italy from Switzerland to Denmark (+Iceland), in localities ranging from 10 m.a.s.l. in Holland to 1300 m.a.s.l. in Switzerland, lacked autosomal sex-determinants (Table 1). Since in these 12 populations sex-determination was based on the presence of *Y* in males only, these populations are referred to as standard populations.

Table 1. *Information on the field populations of* Musca domestica *L. classified as 'standard'*

Years of collection	Origins of samples	No. of samples	Geographic code no. of sites*	No. and types of karyotypes observed $XX♀♀$	$XY♂♂$
1978	Iceland	1	S 1	30	30
1978	Denmark	4	S 2–3–4[2]	115	105
1977–8	Holland	4	S 5–6[r]–7	128	162
1978	Germany	1	S 8	54	85
1980	Switzerland	3	S 9–10–11	81	167

* Geographic references: S 2 N-Jylland, S 3 S-Jylland, S 4 Sjaelland; S 5 Delftland, S 6 Gelderland, S 7 Limburg; S 8 Baden-Württemberg; S 9 Mittelland, S 10 Vaud, S 11 Valais.

[2] Two collections in different sites distant less than 10 Km (S4[2]);

[r] Repeated collections in different years (S6[r]).

(*b*) *Autosomal populations*

All 11 populations were collected in Italy, South of the 44th parallel, in localities near the coast-line and/or below 100 m.a.s.l. (Table 2, Fig. 1). Both sexes lacked *Y* and genetic tests (Scheme 1) repeatedly indicated the presence of autosomal sex-determinants. Each population had at least one *M* homozygous in some of the males indicating the presence of *M* carrier females and so of F. *M* III was present in all the populations, sometimes together with *M* II. Since the position of *M* III was not determined, it is not known whether or not it corresponds with *M* III of the Lab.-1 strain from Florida (Rubini & Franco, 1972).

The term 'autosomal', used here to describe populations lacking *Y*, but having

Table 2. *Information on the field populations of* Musca domestica *L. classified as 'autosomal'*

Years of collection	Origins of samples	No. of samples	Geographical code no. of sites*	No. and types of karyotypes observed $XX♀♀$	$XX♂♂$
1975–6–8	Central Italy	5	A 1–2–3–4–5	129	130
1978	Southern Italy	4	A 6–7–8–9	217	253
1978–9	Sicily	2	A 10–11	88	83

* See Fig. 1 for the geographic references and altitude.

autosomal sex-determinants, replaces 'atypical' used previously (Milani, Rubini & Franco, 1967), because it is more appropriate, since populations lacking *Y* appear widespread in nature.

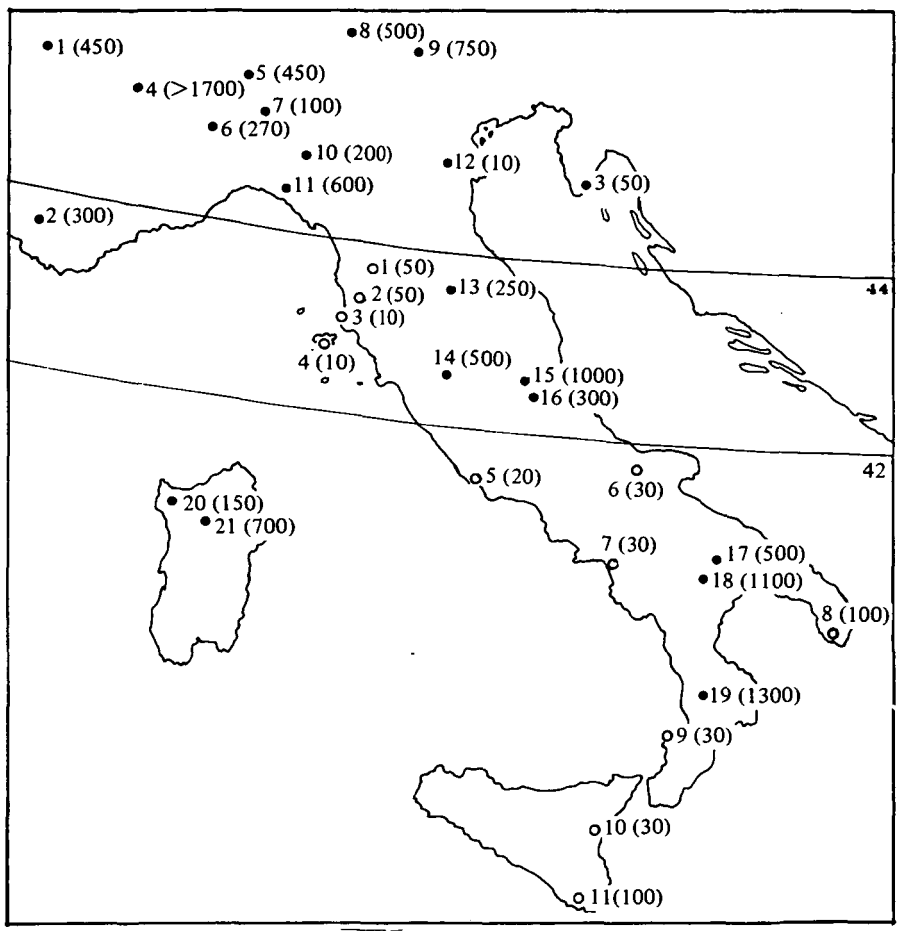


Fig. 1. Geographical distribution and altitude of the sites where autosomal (o) and mixed (•) populations of *Musca domestica* L. have been collected (see Tables 2 and 3).

(c) *Mixed populations*

Twenty-seven of these were Italian, one was from Northern Yugoslavia and two from South Eastern France (Table 3, Fig. 1). All were either from localities North of the 44th parallel or from sites above 250 m.a.s.l. South of this parallel, excepting one (code no. M 20) discussed later.

These populations had at least two different karyotypes in each sex: females were either *XX* or *XY*, males *XY* or *XX* and in two cases *XY* or *YY*. In several populations all the combinations of *X* and *Y*, i.e. *XX*, *XY*, and *YY* were observed

Table 3. *Information on the field populations of* Musca domestica L. *classified as 'mixed'*

Years of collection	Origin of samples	No. of samples	Geographic code no. of sites*	No. of flies examined		No. and types of karyotypes observed (♀♀)			No. and types of karyotypes observed (♂♂)		
				♀♀	♂♂	*XX*	*XY*	*YY*	*XY*	*YY*	*XX*
1980	France	1	M 1	36	87	27	9	0	67	7	13
1975	France	1	M 2	37	49	35	2	0	29	0	20
1980	Yugoslavia	1	M 3	47	69	37	10	0	32	1	36
1976	Northern Italy	7	M 4^{7}	128	92	101	20	7	78	14	0
1975–6	Northern Italy	4	M 5^{2r}	98	178	75	22	1	156	22	0
1978–80	Northern Italy	4	M 5^{2r}	66	94	39	20	7	63	15	16
1975	Northern Italy	1	M 6	35	44	33	2	0	22	0	22
1976–8	Northern Italy	4	M 7^{2r}	92	149	74	18	0	95	1	53
1979	Northern Italy	1	M 8	33	56	23	8	2	47	5	4
1979	Northern Italy	2	M 9^{2}	41	52	39	2	0	43	1	8
1978	Northern Italy	1	M 10	63	72	46	14	3	22	2	48
1978	Northern Italy	1	M 11	49	46	39	10	0	2	0	44
1981	Northern Italy	1	M 12	44	61	35	4	5	24	2	35
1978	Central Italy	1	M 13	54	63	50	1	3	1	4	58
1978	Central Italy	1	M 14	32	72	25	7	0	14	0	58
1979	Central Italy	1	M 15	50	43	43	6	1	24	0	19
1979	Central Italy	1	M 16	37	54	31	5	1	19	2	33
1979	Southern Italy	1	M 17	51	62	48	3	0	1	0	61
1979	Southern Italy	1	M 18	39	25	38	1	0	1	0	24
1977	Southern Italy	1	M 19	56	40	55	1	0	6	0	34
1980	Sardinia	1	M 20	28	96	23	5	0	10	0	86
1980	Sardinia	1	M 21	36	68	34	2	0	6	0	62

* See Fig. 1 for the geographic references and altitude. ^{2} and ^{7}, More collections in different sites distant less than 10 km; ^{r}, Repeated collections in different years; in one case (*M* 5^{2r}) the results obtained in different years are reported separately because this population has shown modifications during this period.

either in females only or in both sexes. This variety of karyotypes indicated the co-existence of the two mechanisms of sex-determination, chromosome *Y* and autosomal sex-determinants (Milani, Rubini & Franco, 1967; Rubini, Franco & Vanossi Este, 1972) and for this reason these populations were called mixed. The results obtained from crosses (Scheme 1) and genetic analysis showed that *M* III is more frequent than *M* II. Moreover in a few samples (code no. M 3, M 20, M 21) the existence of another male determinant still unknown is strongly suspected.

Table 4. *Percentages of XX♂♂ in the 'mixed' populations in relation to the latitude and altitude of the sites of collections*

Latitude	Altitude m.a.s.l.	Geographic code no. of sites*	No. of ♂♂ examined	% of XX♂♂
North of 44th parallel	10 and 50	M 3–12	130	54·61
	From 100 to 270	M 6–7–10	265	46·41
	From 450 to 750	M 1–5–8–9–11	513	16·57
	> 1700	M 4	92	0·00
		Total	1000	27·90
Between 44th and 42nd parallels	From 250 to 500 1000	M 2–13–14–16	238	71·00
		M 15	43	44·19
		Total	281	66·90
South of 42nd parallel	500 and 700 1100 and 1300	M 17–21	130	94.61
		M 18–19	65	89·23
		Total	195	92.82

* See Table 3 and Fig 1 for the references. The site M 20 (Sardinia) is not included; see Discussion and Conclusion.

(*ii*) *Analysis of the geographic location of the different karyotypes in mixed populations*

The distribution of the different karyotypes in the mixed populations and in particular of *XX* males was very heterogeneous, the frequency of the latter ranging from 0 % in samples M 4 and M 5 (1975–6) to 98·32 % in M 17 (Table 3).

To determine if the frequency of *XX* males and the geographical position were correlated, the area containing the mixed populations was divided arbitrarily into three latitudinal zones, and in each zone the male *XX* frequency was analyzed in relation to the altitude of the collection sites.

Table 4 shows that the percentage of *XX* males increases along a cline from North to South through the three zones, considering both the whole populations of each zone and the fractions of populations collected at different altitudes in the three zones. On the other hand, in each zone the percentage of *XX* males decreases as the altitude increases. The overlap between the latitudinal and altitudinal gradients is further illustrated in Fig. 2, in which also the autosomal populations collected in the same latitudinal zones are represented.

It should also be noted that, whereas North of 44th parallel, males in the highest

localities of the Italian Alps (> 1700 m.a.s.l., code no. M 4, Table 3) always had a *Y* chromosome as male determinant either as *XY* or *YY*, thus approaching the karyotype of males of the standard Swiss populations, South of 42nd parallel 94·6 % of the males caught at middle altitude (between 500 and 700 m.a.s.l., code no. M 17–M 21) lacked chromosome *Y*, thus resembling males of the autosomal populations of the coastline, in which *Y* chromosome was never observed.

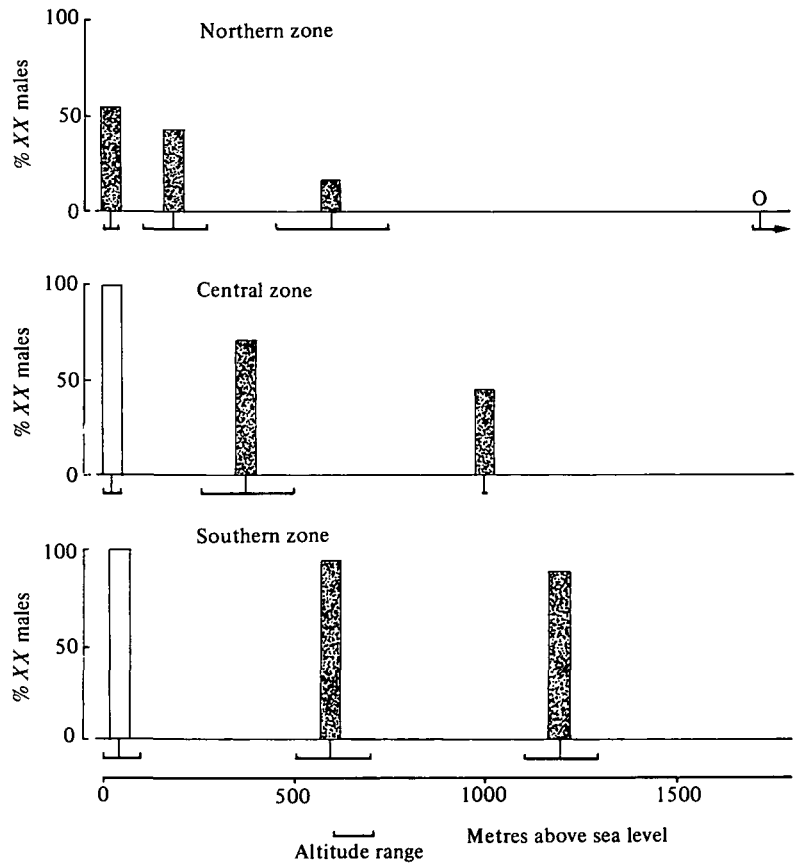


Fig. 2. Comparison of the percentages of *XX* ♂♂ in the three geographical zones where autosomal and mixed populations have been collected. Northern zone is north of 44th parallel. Central zone is between 44th and 42nd parallel. Southern zone is south of 42nd parallel.

(*iii*) *Aneuploidy of chromosome X*

In laboratory strains of *Musca domestica* L. aneuploidy of *X* is common, but the number of *X* chromosomes does not influence sex-determination (Rubini & Palenzona, 1967). In the field populations examined aneuploidy of *X* ranged from 3 to 10 % and the following karyotypes were identified:

XO–XXX ♀♀; *OY–XXY* ♂♂ in standard populations;
XO–OY–XXX–XXY-XYY ♀♀ and ♂♂ in mixed populations;
XO–XXX ♀♀ and ♂♂ in autosomal populations.

These aneuploids collected in the field, like those of laboratory strains, were fully viable and fertile, even *XO* females apparently lethal in Japanese flies (Hiroyoshi 1964; Tsukamoto, Shono & Horio, 1980).

The genetic tests confirmed that chromosome *X* is neutral as regards sex-determination. For this reason flies aneuploid for *X* were added to normal diploids (*XO* = *XX*, *XXX* = *XX*, *OY* = *XY*; *XXY* = *XY*; *XYY* = *YY*) in tables 1–3.

(*iv*) *Polymorphism of chromosome Y*

Both chromosomes *X* and *Y*, clearly recognizable by differences in size, show polymorphism which does not affect sex-determination (Rubini, 1967; Rubini, Franco & Vanossi Este, 1972). Besides, two differently shaped *Y* chromosomes co-existing in the same field population have been described (Rubini, van Heemert & Franco, 1977).

Plate 1 shows some of the new examples of the variability of chromosome *Y* in field populations examined in this work. These morphologically different types of *Y* retain the same value as male determinants in spite of variations in length or arm-ratio.

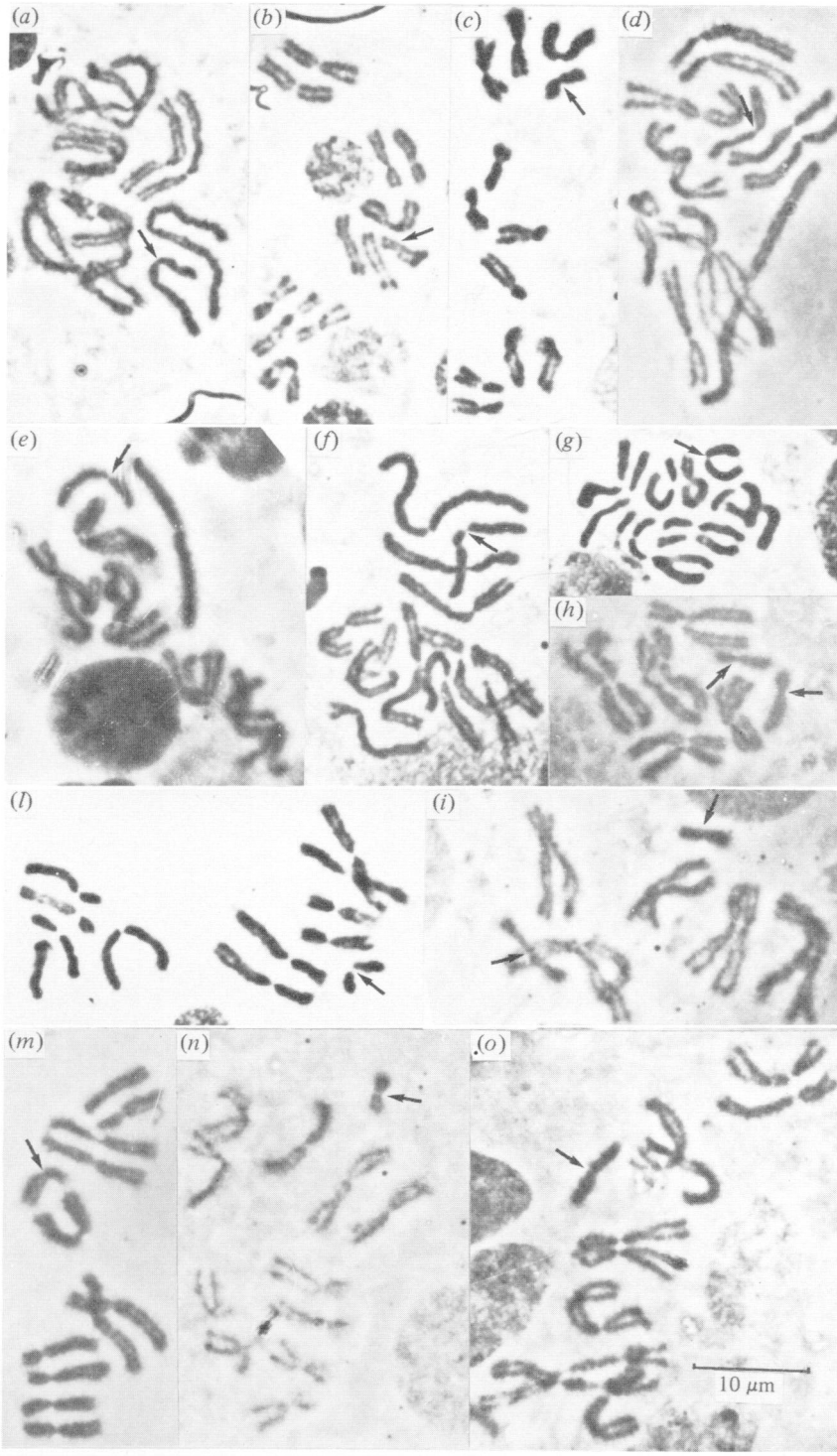
4. DISCUSSION AND CONCLUSION

The results presented above show the existence of a definite gradient in the karyotype of housefly populations of Western Europe along a latitudinal cline from North, where only standard populations were found, to South, where only autosomal populations were present. Clines involving both *M* and *Y* are probably present in North America, as postulated by Bull & Charnov (1977) on the basis of the research of McDonald *et al.* (1975).

In our work there was also strong evidence of an altitudinal cline in the large transition zone in populations of mixed karyotypes, between North and South. The only exception, a mixed population from North Sardinia (M 20, 5 km from the coast and 150 m.a.s.l., Table 3, Fig. 1) could either be due to the late period of collection (October) or micro-climate; this site is very exposed to Northerly winds.

PLATE 1

Polymorphism in length and arms-ratio of the *Y* chromosome in field populations of *Musca domestica* L., documented in gonial mitotic metaphases. (The arrow indicates the centromere of the Y chromosome). (*a*), (*b*) Two *XY* ♂♂ from Iceland (S 1); (*c*), (*d*) Two *XY* ♂♂ from Denmark (S 4); (*e*) *XY* ♂ from Denmark (S 2); (*f*) *XY* ♂ from Germany (S 8); (*g*) *OY* ♂ from Switzerland (S 10); (*h*) *YY* ♀ from Northern Italy (M 4); (*i*) *XY* ♂ from Sardinia (M 20); (*l*) *YY* ♀ from Northern Italy (M 10); (*m*) *XY* ♂ from Central Italy (M 15); (*n*) *XY* ♀ from Southern Italy (M 17); (*o*) *OY* ♀ from Southern Italy (M 17).


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(l)
(m)
(n)
(o)
10 μm

Our research suggests that climate influences both the type and frequency of sexual determinants in housefly populations, but the way this occurs is not known. Climate might either act on survival of the overwintering stage, on the length of development, or adult life span. Since higher temperature increases the number of generations, the localization of autosomal populations in Central and Southern Italy would represent the more advanced stage of a micro-evolutionary process triggered by climate and involving autosomal sex-determinants. This, however, is hard to reconcile with the ubiquity and high mobility (both active and passive, such as by human means of transport) of houseflies, and the complete inter-fertility observed even between specimens of populations as widely separated as those of Iceland and Sicily. Ethological factors such as olfactory stimuli (Rubini, Redi & Franco, 1978) could impede mixing of genotypes of different geographic origins by hindering rapid gene flow between neighbouring populations, and by hampering mating of long distance migrants. However, this is unlikely because there is evidence that the development of the karyological gradient is a dynamic phenomenon probably of recent origin.

Genetic evidence based on detailed research of DDT-resistance, isolation and inheritance of mutants, sex-ratio controls etc. done on houseflies of localities of Central Italy close to Rome between 1954 and 1957 (Milani, 1956, 1961, 1964*b* and personal information) failed to produce results interpretable even *a posteriori* as due to autosomal sex factors. However, the presence of sexually abnormal flies in this area reported by Saccà (1955) may suggest the existence of fractions of the female determinant F, interpreted more recently as one of the possible causes of sexual morphological anomalies (Rubini & Franco, 1968; Milani, 1971). Thus, in spite of the lack of cytological data, there is strong evidence fo believing that 20–25 years ago or more housefly populations in the vicinity of Rome had only *Y* as the male determinant and possibly an unknown proportion of the female determinant F.

Since then the situation has altered, so that by 1978 all the flies collected in this area (Table 2, Fig. 1, code no. A 5) were autosomal and lacked chromosome Y, having instead F, *M* III and *M* II. Furthermore, in a population where both *Y* and F were firstly recorded (Rubini, van Heemert & Franco, 1977), there were no *XX* males in 1975 and 1976, but such males were found in 1978 and 1980 (Table 3, code no. M 5). These observations strongly suggest that male autosomal determinants appeared only recently, while *Y* and F were still present in this area.

Indirect confirmation that variations in the sex-determining mechanism observed by us in European flies might be a dynamic phenomenon is given by Bull & Charnov (1977), who proposed a mathematical model to explain changes in the mechanisms of sex-determination based on the results of Hiroyoshi (1964) and McDonald *et al.* (1975). Bull & Charnov (1977) believe that in the housefly 'the *XY* mechanism is ancestral to the others' and that 'the evolution of *XX* males (...) resulted when the *XX*/*XY* mechanism was invaded by a strong male determiner on autosome III. For some reason, the *XX*, males had higher fitness than the *XY* males, and the *Y* chromosome was selected against'.

If this were true, the populations of Northern Europe would represent the ancestral condition (*XX/XY*), the coastal populations of Central and Southern Italy (*XX/XX*) the new situation and the intermediate populations a transitional phase in the evolutionary process.

This process could have been accelerated by the widespread use of insecticides against houseflies during the last 30–40 years, since it is now possible to interpret most of the observations regarding association between DDT-resistance and sex in the housefly (Milani & Franco, 1959*a*, 1959*b*; Kerr, 1960, 1961; Milani, 1962) as the consequence of linkage between *M* and insecticide resistance genes. Milani (1969) indicated that the association of *M* III with a DDT-resistance gene *kdr*-o would facilitate the fixing of the male factor by DDT-selection, particularly since the presence of F would allow homozygosity of *M* III and *kdr*-o in both sexes. Kerr (1970) described the replacement of *XY* males by *XX* males following selection with DDT in a housefly strain with *M* II linked with DDT-resistance on autosome II. More recently, Rupes & Pinterova (1975) reported linkage between *M* III and a DDT-resistance factor in two Czeck laboratory strains subjected to DDT pressure in field or in laboratory.

Linkage with resitance genes could thus favour autosomal sex-determinants. However, genetic co-adaptation would have had to evolve simultaneously to ensure a normal sex ratio in those populations. This selection could thus be considered to be the 'starter' for genetic change in the Scheme(s) proposed by Bull & Charnov (1977). The appearance and fixation of a female determinant epistatic to *M*, leading to the homozygosity of *M* in both sexes, would be the next step in this evolutionary process.

The polygenic structure of F recognized in a laboratory strain (Rubini, Franco & Vanossi Este, 1972) and in a field population (Rubini, van Heemert & Franco, 1977) may be suspected in a few mixed populations where sporadic intersexes have been observed. Weak F (Milani, 1971) would allow for the presence of heterozygous *M* in females, but genetic co-adaptations, possibly involving minute tandem-duplication or asymmetric crossing-over, could lead to the formation of a strong F epistatic to *MM* or *MY*. This strong F would act as a unitary gene and its fixation might be considered as subsequent to the appearance of *M* as in the case reported by Milani (1969).

This hypothesis, however, does not satisfactorily explain the presumed presence of portions of F without *M* in Italian populations from Central Italy during the 1950s and in two mixed populations (Table 3, M 4; M 5, 1975–76) which had F and *Y* but apparently lacked *M*; these might have represented a transitional phase, as discussed earlier.

The available information does not yet allow us to correlate the variety of sex-determinants in different populations with specific insecticide-resistance genes. The evidence presented here indicates however that both climate and widespread use of insecticides have probably played a considerable part in the micro-evolution of sex-determinants in the housefly in the area studied.

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