Polymorphism of *M* factors in populations of the housefly, *Musca domestica* L., in Turkey

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Summary

M factors, which determine maleness in *Musca domestica*, were found on the second, third, fourth and fifth linkage groups in housefly populations of Turkey. As in European populations, the male-determining factor was more frequently located on linkage group III (M^{III}). Some males homozygous or double heterozygous for *M* factors were identified. Deviations from a 1:1 sex ratio in favour of males, as well as mosaics for somatic marker mutations and sexual mosaics (gynandromorphs), were also observed. The results reveal an extensive polymorphism in the sex-determining system.

1. Introduction

There are three sex-determining systems in houseflies isolated from natural populations (Dübendorfer *et al.*, 1992). In standard populations of *Musca domestica*, sex is determined by a heterosomal mechanism, whereby females are XX and males are XY; the Y carries a male-determining factor, *M* (Rubini & Palensona, 1967). In other populations, *M* factors are located on different autosomes or on the X, with males being heterozygous for *M* (Wagoner, 1969; Rubini & Franco, 1972; Hiroyoshi & Inoue, 1979; Tomita & Wada, 1989). In such populations, no Y chromosome is found. In still other populations, males and females are homozygous for one or more *M* factors but females are heterozygous for a feminizing factor, F^D, one copy of which is epistatic to one or more *M* factors on the Y or on other chromosomes. These three systems have been observed in Japan (Inoue *et al.*, 1983; Tomita & Wada, 1989), in Europe (Franco *et al.*, 1982; Denholm *et al.*, 1985) and in Africa (Denholm *et al.*, 1990).

Polymorphisms for *M* factors have also been reported in several other dipteran species, including *Megaselia scalaris* (Mainx, 1964; Willhoeft & Traut, 1995), *Culex tritaeniorhynchus* (Baker & Sokal, 1976), *Chironomus oppositus* and *Chironomus australis* (Martin *et al.*, 1980; Martin & Lee, 1984).

The objective of this study was to investigate the degree of polymorphism in sex determination in housefly populations in Turkey.

2. Materials and methods

(i) *Wild-type strains*

Population cages were initiated with about 100 flies (males and females) collected between July and August 1992 from 36 different locations in Turkey (see figure 1 in Çakır and Kence, 1996). Flies were caught from different parts of each location to build up a representative sample from the gene pool of each population. The frequency of XX males for each population was determined by cytological examination in the first generation (Çakır & Kence, 1996).

(ii) car;bwb;cyw *marker strain*

The *car;bwb;cyw* laboratory strain is homozygous mutant for carnation eye (*car*) on the second chromosome, brown body (*bwb*) on the third chromosome and curly wing (*cyw*) on the fourth chromosome.

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Table 1. *Summary of the F1 results of all single-pair crosses between females from the* car;bwb;cyw *strain and males from the original populations* (*Giresun, A; Trabzon, B; Kayrak, C; İzmit, D; İskenderun, E; Simav, F; Balıkesir, G; Polatlı, H*)

Sex ratio	A	B	C	D	E	F	G	H	p	m	f
All ♂	9	12	0	0	1	0	1	0	23	1596	0
♂ > ♀	7	8	0	0	8	1	0	0	24	1399	282
♂ < ♀	0	0	2	1	0	0	0	0	3	109	196
♂ = ♀	11	4	24	11	7	16	8	19	100	3809	3744
Total	27	24	26	12	16	17	9	19	150	6913	4222

p, total number of single-pair crosses; m, total number of males; f, total number of females.

These are all recessive mutations, and this strain has a standard sex-determining mechanism with XX females and XY males.

(iii) *Laboratory conditions*

All crosses were done at constant illumination (60 W fluorescent lamp), 25±3 °C and 50–70% relative humidity. Eggs were collected from population cages, and cultures with 350 eggs/100 g larval medium (Kence & Kence, 1992) were prepared for each population cage in each generation. Adult flies were kept in 30 × 30 × 30 cm population cages and fed with powdered milk, cube sugar and water.

(iv) *Chromosomal localization of* M *factors*

To find out the chromosomal localization of *M* factors, linkage analysis was carried out by using the *car;bwb;cyw* mutant stock. Techniques and procedures for analysing sex determinants in field strains have been described by Denholm *et al.* (1983, 1985). The linkage of *M* factors is determined by single-pair test crosses between standard XX females marked with visible recessive mutations on the autosomes and males from the field populations. Recombination in males is rare or absent (Rubini *et al.*, 1980). Eight populations with high frequencies of XX males (and low frequencies of XY males) were chosen for these experiments, namely Giresun (#15, 100% XX males), Trabzon (#16, 100%), Kayrak (#5, 90%), İzmit (#23, 100%), İskenderun (#28, 76%), Simav (#34, 72%) and Balıkesir (#33, 75%) (for geographical locations see figure 1 in Çakır & Kence, 1996). The Polatı population (#36) with 97% XY males was used for the control experiments as a standard population.

To find out the number and location of *M* factors, 20–30 single-pair crosses were set up between females from the *car;bwb;cyw* strain and males from each of the eight chosen population cages (A–H) in the second generation after collection. All the F1 flies from 150 successful crosses were wild-type as expected (Table 1). Three to five heterozygous males were randomly taken from F1 progenies of the 150 crosses. The chromosomal localization of *M* factors was revealed by single-pair backcrosses between these F1 males taken from each sample and females from the *car;bwb;cyw* stock. Sex and phenotypes of the F2 individuals were recorded for each testcross.

3. Results

(i) *F1 results of all single-pair crosses*

An unexpected result of the single-pair crosses was a frequent deviation from a 1:1 sex ratio in F1 individuals (Table 1). The number of males resulting from crosses between *car;bwb;cyw* females and males of A, B and E populations was significantly higher than the number of females. In experiments involving males from C, D, F, G and H populations, on the other hand, the sex ratio was mostly 1:1. In some rare cases, a single male produced significantly more daughters than sons. For example, a single male from Kayrak had 20 sons and 43 daughters; another had 16 sons and 38 daughters.

(ii) *F2 results of the Giresun* (*A*) *strain*

In the F2 results of the Giresun (A) strain only one male (numbered as A9) showed sex-linked inheritance for the third chromosome yielding *bwb* daughters and bwb^+ sons (Table 2). Three males (for instance, A12) produced *bwb* daughters, but also sons with all possible eight phenotypes including *bwb*. Sex-linked inheritance for *car;bwb;cyw* was not found in the F2 progenies of other males. In this strain, sex ratios

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Table 2. *Some of the F2 results of single pair backcrosses between the heterozygous F1 males from the Giresun (A), Trabzon (B), Kayrak (C) and Simav (F) populations and females from the* car;bwb;cyw *strain*

Localities:	Giresun (A)						Trabzon (B)							Kayrak (C)								Simav (F)					
No. of males used:	A9		A12		A26		B10			B18		B26		C14		C18		C24 (a)		C24 (b)		F3		F4		F17	
F2 phenotypes:	♂	♀	♂	♀	♂	♀	♂	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
+;+;+	34	0	5	0	42	14	21	0	1	29	0	40	17	19	0	68	0	103	0	18	18	46	0	32	0	16	24
+;+;*cyw*	20	0	3	0	13	3	2	11	4	7	25	31	18	20	0	41	0	74	0	4	0	37	0	29	0	14	18
+;*bwb*;+	0	24	7	3	8	16	27	0	0	34	0	37	19	0	22	0	34	0	59	0	21	0	41	0	36	10	27
+;*bwb*;*cyw*	0	18	4	5	3	2	6	9	6	13	24	26	25	0	19	0	22	0	50	0	1	0	18	0	22	14	12
car;+;+	27	0	6	0	6	6	24	0	0	29	0	37	8	13	0	24	0	41	0	2	0	36	0	23	0	9	11
car;+;*cyw*	22	0	11	0	0	5	4	16	9	9	24	45	17	17	0	17	0	43	0	3	0	40	0	25	0	15	17
car;*bwb*;+	0	18	4	8	2	3	11	0	0	26	0	38	24	0	11	0	12	0	47	0	3	0	23	0	24	7	6
car;*bwb*;*cyw*	0	20	5	2	4	1	5	10	7	10	9	21	19	0	15	0	16	0	19	0	1	0	21	0	18	4	5
Total	103	80	45	18	80	50	100	46	27	115	43	275	144	69	67	150	84	261	175	27	44	159	103	109	100	89	120
M factor	M^{III}		$M^{III}+M^{a}$				$M^{IV}+M^{a}$			$M^{IV}+M^{a}$		M^{a}		M^{III}		M^{III}		M^{III}		M^{III}		$M^{III}+M^{a}$		M^{III}			
Sex ratio	♂ = ♀		♂ > ♀		♂ = ♀		♂ > ♀			♂ > ♀		♂ > ♀		♂ = ♀		♂ > ♀		♂ > ♀		♂ = ♀		♂ > ♀		♂ = ♀		♂ = ♀	

Summary of the F1 results was given in Table 1, columns A, B, C and F. [a] Chromosomal localization unknown.

Table 3. *Some of the F2 results of single-pair backcrosses between heterozygous F1 males from İzmit and females from the* car;bwb;cyw *strain*

Locality:	İzmit (D)																	
No. of males used:	D1 (a)		D1 (b)		D6		D17 (a)		D17 (b)		D17 (c)		D18 (a)		D18 (b)		D25	
F2 phenotypes:	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
+;+;+	5	0	19	0	26	0	47	0	11	2	29	0	61	0	24	8	57	0
+;+;*cyw*	11	0	22	0	25	0	42	0	20	2	19	0	38	0	26	3	61	0
+;*bwb*;+	0	9	22	0	19	0	38	0	5	4	0	16	28	0	21	9	41	0
+;*bwb*;*cyw*	0	16	22	0	22	0	19	0	7	0	0	19	27	0	13	4	34	0
car;+;+	4	0	0	15	0	25	0	33	0	6	8	0	0	39	0	20	0	49
car;+;*cyw*	9	0	0	22	0	28	0	29	0	12	14	0	0	39	0	12	0	28
car;*bwb*;+	0	5	0	28	0	21	0	41	0	10	0	0	0	22	0	6	0	13
car;*bwb*;*cyw*	0	7	0	22	0	18	0	21	0	4	0	6	0	13	0	3	0	9
Total	29	37	85	87	92	92	146	124	43	40	70	41	154	113	84	65	193	99
M factor	M^{III}		M^{II}		M^{II}		M^{II}		M^{II}		M^{III}		M^{II}		M^{II}		M^{II}	
Sex ratio	♂ = ♀		♂ = ♀		♂ = ♀		♂ = ♀		♂ = ♀		♂ = ♀		♂ = ♀		♂ = ♀		♂ > ♀	

Summary of the F1 results is given in Table 1, column D.

deviated significantly from 1:1 in favour of males in the offspring of 7 males (for instance, A12). Test-crosses of F1 males also produced a small proportion of somatic mosaics having both mutant and wild-type tissues.

(iii) *F2 results of the Trabzon (B) strain*

Only the F2 progenies of males of the Trabzon (B) strain numbered as B10 and B18 gave results showing partial sex-linked inheritance for the fourth chromosome, producing *cyw* daughters and males with all possible eight phenotypes including rarer *cyw* flies (Table 2). If there was a single sex-determining factor on the fourth chromosome (mutant marker is *cyw* for this chromosome), none of the males in the F2 generation should be *cyw*. The other unexpected result was the occurrence of intersexes only among *cyw* individuals (Table 2, B10). Sex-linked inheritance was not observed in the F2 of the other males, and the F2 results of six males showed significant deviations in sex ratio in favour of males. Some somatic mosaics and gynandromorphs, which are partly male and partly female, were also observed.

(iv) *F2 results of the Kayrak (C) strain*

In general, the females in F2 generations of the Kayrak (C) strain had brown body colour while all males had normal body colour (Table 2), indicating that males in this population were heterozygous for an *M* factor on the third chromosome. In addition, one male of C18 and C24 gave sex ratios that deviated from 1:1 in favour of males, and some somatic mosaics for marker genes were also observed.

(v) *F2 results of the İzmit (D) strain*

There were four different modes of inheritance in terms of sex determination among F2 progenies of 25 males of the İzmit (D) strain test-crossed (Table 3). Backcrosses of 5 F1 males (D1 b, D6, D17 a, D18 a, and D25) revealed the presence of an *M* factor on the second chromosome segregating from *car*. Two males (D1 a and D17 c) gave results indicating the presence of an *M* factor on the third chromosome. The results in the F2 generation of 3 males (for instance D18) indicated an *M* factor on the second chromosome, but in D18 b, unexpected females with normal eye colour appeared. Phenotypic characteristics of F2 progenies of males numbered as D17 indicated that D17 was heterozygous for *M* factors on both the second and third chromosomes, but again females with normal eye colour were produced when *M* was on the second chromosome. The sex ratios were 1:1 except for the D25 progenies. A small number of somatic mosaics for marker genes were observed in F2 progenies.

(vi) *F2 results of the İskenderun (E) strain*

In the İskenderun (E) strain, 6 males (for instance E11) had an *M* factor on the second chromosome, and E8 was heterozygous for two *M* factors located on both the third and the fourth chromosomes (Table 4). The phenotypic characteristics of progenies of 2 males (for instance E3) did not show any clear mode of inheritance for sex determination, but a significant increase in number of males and flies with different wing shape was observed. On the other hand, 2 males (for instance E5) showed two different modes of inheritance for sex determinants. The F2 progenies of some F1 males indicated the location of an *M* factor on the third chromosome (E5 a, E8 a), but the other F1 males gave results indicating the effect of both the second and the third chromosomes in the sex determination of these F2 progenies (E5 b). E4 had *M* factors on both the second and the fourth chromosome. In this strain, M^{II}, M^{III} and M^{IV} were identified, and some sex ratios deviated significantly from 1:1. A small proportion of somatic mosaics for marker genes and gynandromorphs were also observed in F2 progenies.

(vii) *F2 results of the Simav (F) strain*

All female progenies of the F2 generation of 12 males of the Simav (F) strain (for instance F4) had *bwb* colour indicating an *M* factor on the third chromosome (Table 2), but the other males tested (F17) did not show sex-limited inheritance for either the second, third or fourth chromosomes. Some sex ratios deviated significantly from 1:1 in favour of males.

(viii) *F2 results of the Balıkesir (G) strain*

All males of the Balıkesir (G) strain tested had an *M* factor on the third chromosome (data not given), and the F2 progenies of only one male showed a significant excess of males.

(ix) *F2 results of the Polatlı (H) strain*

The same procedure was followed in the Polatlı (H) strain to see if there was sex-limited inheritance of any phenotypes within the F2 progenies indicating the existence of an *M* factor on the second, third or fourth chromosomes. All the single-pair test-crosses indicated that there was no *M* factor on these chromosomes, as

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Table 4. *Some of the F2 results of single-pair backcrosses between the heterozygous F1 males from İskenderun and females from the* car;bwb;cyw *strain*

Locality:	Iskenderun (E)																							
No. of males used:	E3			E4 (a)		E4 (c)		E4 (d)		E4 (e)		E4 (f)		E5 (a)		E5 (b)		E7		E8 (a)		E8 (b)		E11	
F2 phenotypes:	♂	♂♀	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
+;+;+	0	0	0	10	0	19	0	20	0	27	0	19	0	30	0	23	0	10	16	31	0	22	0	22	0
+;+;*cyw*	9	8	0	9	0	26	0	0	22	8	0	12	0	13	0	4	0	10	8	0	21	29	0	10	0
+;*bwb*;+	9	0	0	8	0	1	0	37	0	26	0	27	0	0	35	12	0	6	11	25	0	0	19	5	0
+;*bwb*;*cyw*	10	0	0	3	0	2	0	0	22	10	0	2	0	0	20	8	0	3	3	0	21	0	12	15	0
car;+;+	0	0	0	3	0	0	12	10	0	0	0	0	0	12	0	14	0	6	9	22	0	12	0	0	9
car;+;*cyw*	8	0	8	0	5	0	12	0	18	0	21	0	13	10	0	8	0	7	4	0	23	24	0	0	8
car;*bwb*;+	10	0	0	0	0	0	3	9	0	0	0	0	0	0	12	0	16	4	7	12	0	0	12	0	4
car;*bwb*;*cyw*	0	0	8	0	0	0	3	0	6	0	12	0	0	0	12	0	10	3	3	0	12	0	6	0	2
Total	46	8	16	33	5	48	30	76	68	71	33	60	13	65	79	69	26	103	61	90	77	77	49	52	53
M factor	$M^{II}+M^{a}$			$M^{II}+M^{a}$		M^{II}		M^{IV}		$M^{II}+M^{a}$		$M^{II}+M^{a}$		M^{III}		$M^{II}+M^{a}$		M^{a}		M^{IV}		M^{III}		M^{II}	
Sex ratio	♂ > ♀			♂ > ♀		♂ = ♀		♂ = ♀		♂ > ♀		♂ > ♀		♂ = ♀		♂ > ♀		♂ > ♀		♂ = ♀		♂ > ♀		♂ > ♀	

Summary of the F1 results is given in Table 1, column E. [a] Chromosomal localization unknown.

expected because the frequency of XX males for this strain was only 3%. There were male and female individuals with eight possible phenotypes, consistent with the presence of a Y chromosome in these males. Only 2 of the F2 cases showed a significant excess of males.

4. Discussion and conclusions

As stated in Section 1, several sex-determining systems have been found in strains of *Musca domestica*. Our data reveal an extensive polymorphism of *M* factors in field populations collected in Turkey. In many populations, males proved to be homozygous for *M* factors, or heterozygous for more than one *M* factor. Such males were identified by test-crosses because they produced exclusively sons or a significant surplus of sons. Homozygosity for *M* necessarily implies the presence of an epistatic female-determining factor in the females of such a population. Test-crosses with some females in fact confirmed that they carried the expected epistatic F^D factor on the fourth chromosome (Çakır, unpublished data). Simple heterozygosity for a single *M* factor was also observed.

Very rarely, a single male generated more daughters than sons (3 males in Table 1). This is not expected since a single male should be at least heterozygous for one *M* factor and should thus produce a sex ratio of 1:1. We see three possible explanations: (i) meiotic drive, so that the number of sperm with an *M* factor is significantly reduced relative to sperm without *M*; (ii) the chromosome with the *M* factor confers a dominant disadvantage on its carriers, causing many of them to die; and (iii) a weak *M* factor that allows some carriers to develop as females or intersexes. Such weak, i.e. not fully penetrant, *M* factors have recently been described (Schmidt *et al*., 1997).

What are the factors responsible for the observed polymorphism, and how is such a polymorphism maintained? Many investigators have reported that climatic and geographic factors favour the invasion of autosomal sex-determining factors into housefly populations having a standard sex determination (Franco *et al*., 1982; Denholm *et al*., 1983, 1985; Tomita & Wada, 1989). According to these authors, a micro-evolutionary process might be initiated and increase due to climatic influences. Frequent usage of insecticides has also been suggested as a cause for invasion of autosomal sex-determining factors in housefly populations. This situation was explained by the linkage between autosomal male-determining factors and insecticide resistance genes. A few major genes located on the second, third and fifth chromosomes control resistance to insecticides in the housefly (Hiroyoshi, 1980; Franco *et al*., 1982; Takada *et al*., 1990; Kence & Kence, 1992). Transposition of *M* factors mediated by transposable elements has also been suggested as a cause of *M* factor polymorphism (Green, 1980; Wilson, 1993). There is need for molecular data to explain the mechanism of *M* factor polymorphism of the housefly in field populations.

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