

Competition Between Strains With Autosomal and Standard Sex–determining Mechanisms in the Housefly (*Muscidae*)

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Abstract: Experimental cages started with a mixture of flies at three different ratios (1:9, 5:5, 9:1) from populations having heterosomal and autosomal sex–determining mechanisms were cytogenetically examined over six generations at two different temperatures (25 °C and 35 °C). In general, there was an increase in the frequency of XX males in all competitive population cages. Abnormal testes and intersex appearance, frequent X chromosome aneuploidies and deviation from a 1:1 sex ratio were observed.

Key Words: *Musca domestica* L. Competition, cytogenetics, autosomal sex–determining mechanism, evolution.

Karasinekte (*Muscidae*) Otozomal ve Standart Eşey Belirleme Mekanizmalı Irklar Arasında Yarış

Özet: Otozomal ve heterozomal eşey belirleme mekanizmalarına sahip populasyonlardan, üç farklı oranda (1:9, 5:5, 9:1) sineklerin karışımı ile başlatılan iki farklı ısıdaki (25 °C ve 35 °C) deneysel kafesler, altı generasyon boyunca sitogenetik olarak araştırıldı. Genel olarak bütün populasyon kafeslerinde XX erkek frekansında bir artış vardı. Anormal testisler, interseksüel görünüş, sık X kromosomu aneuploidisi ve 1:1 eşey oranından sapmalar gözlemlendi.

Anahtar Sözcükler: *Musca domestica* L., yarış, otozomal eşey belirleme mekanizması, evrim.

Introduction

It is known that in housefly populations, sex is generally determined by a heterosomal mechanism (XX females and XY males) (1, 2). However, researchers from different countries suggest that autosomal sex–determination has been rapidly spreading and replacing the standard heterosomal system (3, 4, 5, 6). This increase in the frequency of autosomal sex–determination in natural housefly populations has been found to be related to geographical altitude and latitude in European populations (3, 7, 8). Climatic factors and widespread usage of insecticides have been suggested as the reason for the rapid invasion of autosomal sex–determination factors in housefly populations in different parts of the world (9, 10, 11). The most frequently mentioned chromosomes having both autosomal sex determining factors and resistance genes to insecticides are the second and the third chromosomes. The other explanation of why non–standard genotypes are being favoured by selection is that an invading male determinant creates new, competitively superior genotypes (10, 12). Transposition

of male determining factors (*M*) mediated by one or more transposable elements has been suggested as a cause of autosomal *M* factor polymorphism (13).

The objective of this study was to compare the autosomal and standard sex–determining mechanisms in hybrid populations at different ratios and temperatures.

Materials and Methods

Strains of houseflies. Cytogenetic examination has shown the XX male frequency in the Trabzon and Izmit populations to be 100%. The Polatlı population only had 3% XX males (14) and thus was considered to be of the standard heterosomal type.

Laboratory conditions. Populations were reared at constant 24h illumination (60 watt fluorescent lamp), 25±3 °C temperature (except the competitive populations reared at 35±3 °C). Egg were collected from the population cages, and two rearing vials containing 350 eggs/100 g larval medium were prepared for each population over six generations. Adult flies were kept in

30x30x30 cm population cages and fed with powdered milk, cube sugar and water.

Experimental procedures. Experimental hybrid cages were started with a mixture of flies at three different ratios (1:9; 5:5; 9:1) from populations having heterosomal and autosomal sex-determining mechanisms. Two identical sets of population cages were prepared by a mixture of flies from Trabzon (T): Polatlı (P) and İzmit (I): Polatlı (P). One set of population cages was kept at $25\pm 3^{\circ}\text{C}$ and the other set was maintained at $35\pm 3^{\circ}\text{C}$. All population cages were initiated with 30 pairs of virgin adults. At every generation, emerged flies were counted and sex ratio was computed and the 25–30 males were taken from each population cage and examined cytogenetically to find out the ratio of XX males.

Cytogenetic examination. Cytological analysis consisted of microscopic examination of spermatogonial cells of the first meiotic divisions in the testes (15). Five pairs of autosomes and one pair of heterosomes (sex chromosomes) were identified according to size and centromere position (16), and photographs were taken using Kodak ASA 400 film. The preparations were sealed with nail polish for re-examination. Images were magnified 500 times for chromosomal identification in order to determine the frequency of XX males from each population cage.

Results

The results of cytogenetic examinations of the populations in competition reared at 25°C and 35°C are given in Table 1. The values in parentheses show an increased percentage of XX males in competing populations over six generations. The following karyotypes were identified in males: XY, XX, OY, XO, XXY, XXXY, XXX (Figure 1). The number of aneuploid males was slightly higher in the populations reared as 35°C (7.81%) than in the populations reared at 25°C (5.75%), but the difference was not significant. In general, there was an increase in the frequency of XX males in all population cages (except those started with a 5I:5P ratio at 25°C) over six generations.

Testes with abnormal formation were observed during dissection for cytogenetic examination (Figure 2). Some of these abnormal testes had ovarium-like tissues or reduced size. The presence of intersex individuals was also observed in these hybrid populations. The populations prepared with Trabzon and Polatlı strains

produced more intersex individuals than the populations prepared with İzmit and Polatlı strains. There were more intersex individuals reared at 25°C than at 35°C , and the number of intersex individuals decreased over the generations at both 25°C and 35°C . Variations in the sex ratio were random.

Discussion and Conclusion

Latitudinal and altitudinal effects on the distribution of XX male frequency have been reported for housefly populations in Europe (3, 4, 8). Franco et al. (3) suggested that climate influences both the type and frequency of sexual determinants in housefly populations, and that it might affect the survival of the overwintering stage, the length of development or the adult life span. According to them, 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 by climate and involve autosomal sex-determinants. The mobile genetic elements have the potential to increase variability in a gene pool by acting as mutagenic agents. Green (13) has suggested that the *M* factors of different loci may be homologous in ancestry, and are capable of being transposed to different sites. According to Hickey's model (17), a transposon that causes sex-determination in the host would favour its own spread. Among eukaryotes, it is also known that mating type in yeast is controlled by a transposable genetic element (18). Some environment might produce a selective advantage for mutator activities associated with these elements (19, 20). The idea here is that the progeny of dysgenic crosses experienced very high mutation frequencies and some of the resulting mutants were selected, thus fixing the respective transposable elements. In order to check these possibilities, the effects of environmental temperature, the competitive differences of Trabzon and İzmit populations and the ratio of non-standard: standard populations were tested. The direction of change in sex-determining mechanisms was found in favour of autosomal factors in these populations. There was an autosomal advantage in competition between flies with autosomal and heterosomal sex-determining factors (Table 1). The populations started with Trabzon and Polatlı flies showed more XX male advantage than the populations started with İzmit and Polatlı flies. Cytogenetically examined aneuploidy of X in males were XXX, XXY, XXXY, XO, YO (Figure 1) in these competitive populations, but flies without at least one X or Y were

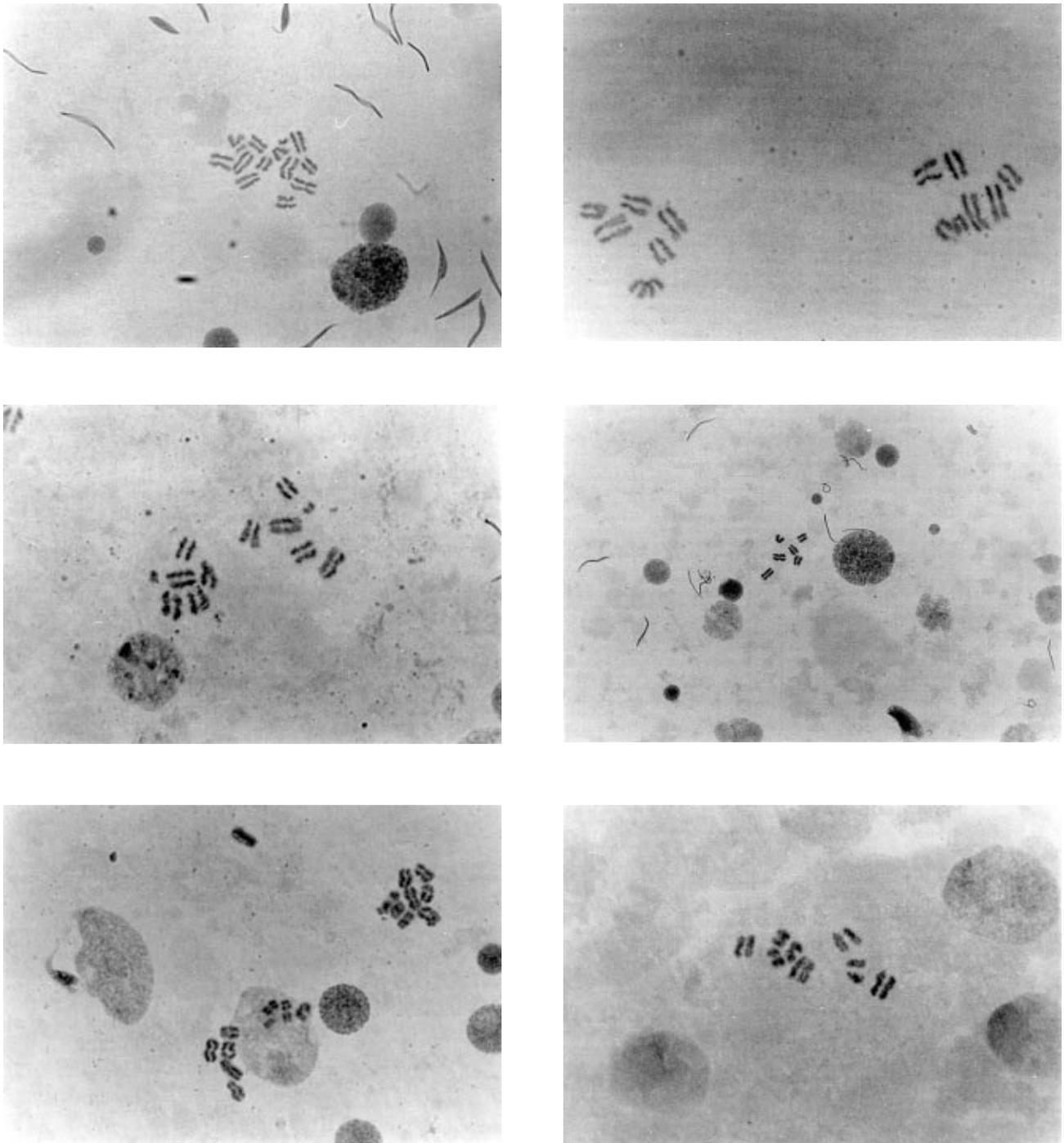


Figure 1. Testis squash meiotic preparations from houseflies having XY(a), XX(b), YO(c), XO(d), XXXY(e) and XXX(f) sex chromosomes. Magnification: 100X5.

never observed. As confirmed by many investigators (3, 10), chromosome X is neutral with regard to sex-determination, but the obligation of having at least

one of X or Y for all flies examined indicated some vital function of sex chromosomes. Testes with abnormal formation (Figure 2) in hybrid populations (Trabzonx

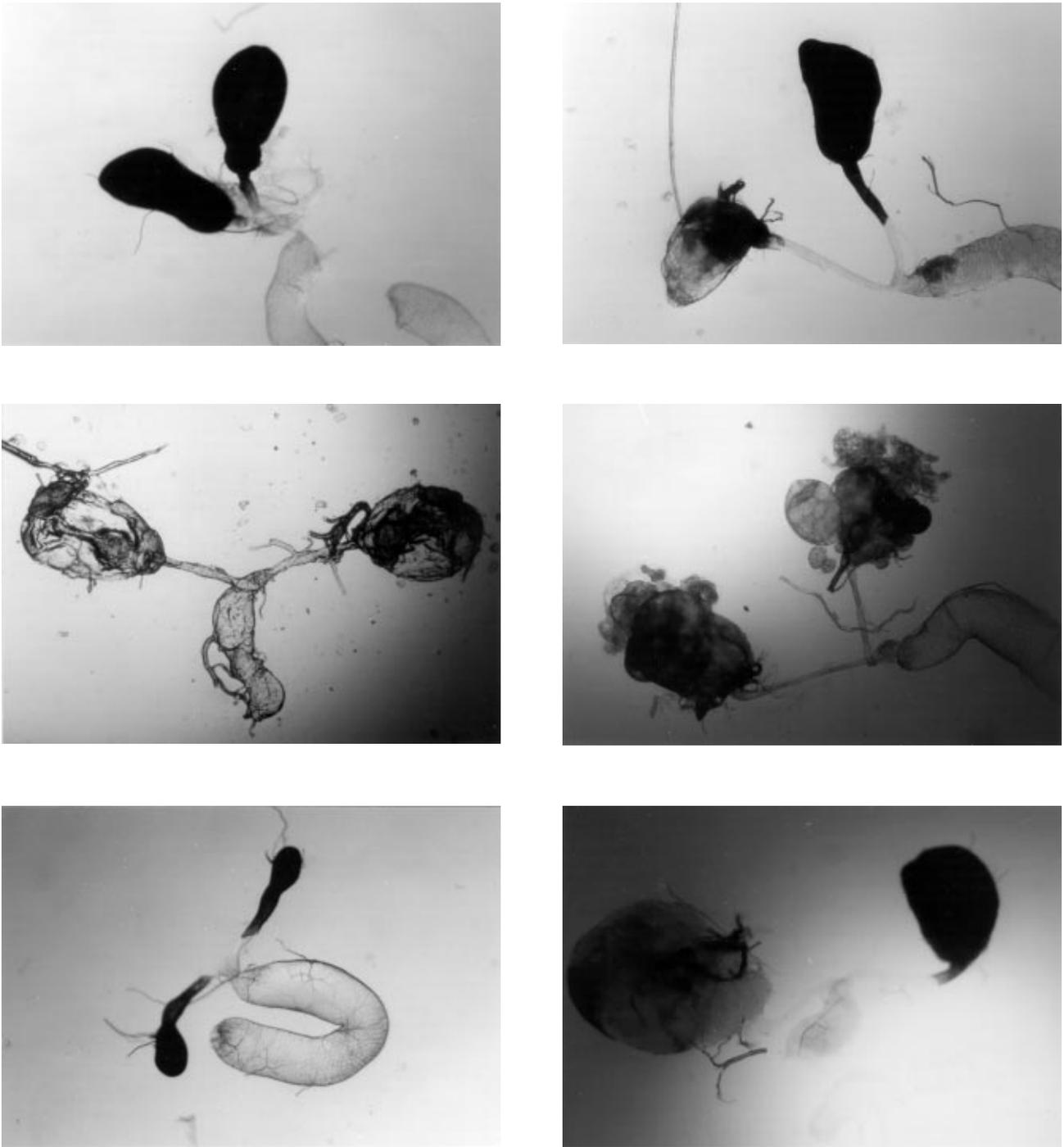


Figure 2. A pair of normal testes (a) and the abnormal testes (b-f) of houseflies. Magnification: 10X5.

Polatlı), especially those reared at 35 °C, are similar to the abnormal gonads of dysgenic *Drosophila* (25). Observation of abnormal gonads, intersex appearance, many cases of aneuploidy of X chromosome and strong

deviations from the 1 male: 1 female sex ratio (22) suggest the possible existence of transposons in the housefly populations of Turkey.

Table 1. The results of cytogenetic examinations of the populations in competition reared at 25 °C and 35 °C. The values in parentheses show the percentage of XX males. T: Trabzon, I: Izmit, P: Polatlı.

	1T: 9P		5T:5P		9T:1P		11:9P		5I:9P		9I:1P	
	25 °C	35 °C 25 °C	35 °C	25 °C	35 °C							
1 st Generation	12XX (50%)	1XX (9.1%)	18XX (72%)	14XX	24XX (85.7%)	16XX (84.2%)	2XX (12.5%)	6XX (30%)	7XX (87%)	16XX (57.1%)	16XX (%)	15XX (88.2%)
	12XX	10XY	7XY	(66.6%)	4XY	3XY	14XY	14XY	1XY	12XY	9XY	7XY
	3Y0			7XY		1XXX	1Y0	1Y0				
2 nd Generation	27	11	25		28	20	17	22	8	28	25	17
	11XX (57.8%)	1XX (4%)	18XX (69.2)	8XX (57.1%)	15XX (83.3%)	14XX (85.7%)	9XX (50%)	9XX (34.6%)	18XX (69.2%)	2XX (10%)	14XX (60.8%)	14XX (60.8%)
	8XY	24XY	8XY	6XY	3XY	2XY	9XY	17XY	8XY	18XY	9XY	9XY
3 rd Generation	1Y0	1Y0				4Y0		1Y0		3X0		3XXY
												2XXX
	20	26	26	14	18	20	18	27	26	23	23	29
4 th Generation	12XX (63.8%)	6XX (33.3%)	14XX (66.6%)	17XX (85%)	22XX (81.5%)	17XX (85%)	11XX (45.8%)	8XX (33.3%)	13XX (46.4%)	4XX (12.2%)	25XX (86.2%)	15XX (68.2%)
	7XY	12XY	7XY	3XY	5XY	3XY	13XY	16XY	15XY	29XY	4XY	7XY
	8X0	1X0	2X0	1XXX	2X0	3X0	6X0	1X0	4X0			1XXY
5 th Generation	4Y0	3Y0				1X0		1Y0	2XXY			1XXX
						1XXX						
	31	22	23	21	29	25	30	26	34	34	29	24
6 th Generation	20XY (74%)	17XX (51.5%)	14XX (50%)	21XX (85%)	25XX (89.3%)	14XX (87.5%)	8XX (33.3%)	8XX (27.6%)	10XX (32.3%)	10XX (30.8%)	35XX (100%)	16XX (55.2%)
	7XY	16XY	14XY	4XY	3XY	2XY	16XY	16XY	21XY	23XY	0XY	13XY
	1X0	2Y0			1Y0		2Y0	2Y0	1X0	2Y0		3X0
7 th Generation					1X0		5X0			1XXY		1XXX
	28	35	28	25	30	16	31	30	32	36	35	33
	22XX (78.5%)	21XX (70%)	17XX (62.9%)	22XX (78.5%)	19XX (86.3%)	25XX (100%)	13XX (54.1%)	7XX (23.3%)	4XX (28.5%)	12XX (34.2)	30XX (100%)	22XX (88%)
8 th Generation	6XY	9XY	10XX	6XY	3XY	0XY	11XY	23XY	10XY	23XY	0XY	3XX
	1Y0	3Y0			1Y0		1Y0	6Y0	1Y0			4XXX
	1X0											1XXY
9 th Generation	30	33	27	28	23	25	25	36	15	35	30	30
	22XX (84.5%)	15XX (60%)	15XX (60%)	19XX (65.5%)	22XX (88%)	13XX (100%)	8XX (40%)	6XX	10XX (33.3%)	10XX (47.6%)	30XX (100%)	29XX (100%)
	4XY	10XY	10XY	10XY	3XY	0XY	12XY	(33.%)	20XY	11XY	0XY	0XY
10 th Generation		2Y0		2X0		5X0	1Y0	12XY	2XY	1XXY		1XXY
		2X0		1XXY			1XXY	1Y0				
	26	29	25	32	25	18	23		32	22	30	30

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