Tr. J. of Zoology
23 (1999) 73–77

Two New Sex Determining Factors (M^V, F^D) in Housefly, (*Musca domestica*) Populations in Turkey

Şükran ÇAKIR
Ankara University, Faculty of Veterinary (Genetics), 06110 Ankara-TURKEY

Received: 20.03.1998

Abstract: In most strains of the common housefly, *Musca domestica,* sex is genetically determined by the presence or absence of a Y chromosome with a dominant male determining factor, *M.* Standard trains have an XX–XY mechanism. In certain laboratory and natural populations, however, neither females nor males have the Y chromosome. These non–standard populations have autosomal mechanisms for sex determination. In the present study, the existence of two new autosomal sex determining factors, M^V and F^D, in housefly populations in Turkey was determined.

Key Words: *Musca domestica,* sex determination, M factor, F^D factor.

Türkiye Karasinek, *Musca domestica*, Populasyonlarında İki Yeni Eşey Belirleyici (M^V, F^D) Faktörü

Özet: Karasineğin, *Musca domestica*, birçok ırkında eşey genetik olarak dominant bir erkeklik belirleme faktörüne, *M*, sahip Y kromozonunun olup olmaması ile belirlenir. Standart ırklar XX–XY mekanizmasına sahiptir. Bununla beraber, standart ırklar yanında, bazı laboratuvar ve doğa populasyonlarında, ne erkek ne de dişiler Y kromozomuna sahiptir. Bu standart olmayan populasyonlar otozomal eşey belirleme mekanizmalarına sahiptir. Bu çalışmayla Türkiye karasinek populasyonlarında iki yeni otozomal eşey belirleme faktörünün, M^V and F^D, varlığı gösterildi.

Anahtar Sözcükler: *Musca domestica*, eşey belirleme, M faktörü, F^D faktörü.

Introduction

Different animal groups exhibit a surprising diversity of sex determination systems. In the class of insects alone, sex can be determined by Y chromosomal, or by autosomal factors (*M*–bearing chromosomes), by the ratio of X chromosomes to autosimes, by haploidy versus diploidy, by maternal effects or by environmental factors (1, 2). The housefly (*Musca domestica*) is known as an insect vector of disease, and for this reason, it is genetically well analyzed (3, 4, 5). Many investigators have confirmed that the diploid chromosome number in the standard housefly is 12, consisting of 5 pairs of autosomes and one pair of sex chromosomes (4, 6, 7, 8, 9). Sex is determined by the presence or absence of the Y–chromosome which carries a dominant male determiner, Y^M.

In recent years, investigations have been performed on sex determination of *Musca,* and have led to the discovery of different sex determination mechanisms (polymorphisms) within this species. In certain laboratory and natural populations, however, neither females nor males have the Y chromosome. Instead, these males carry an autosomal male–determining factor, A^M, at different linkage groups (10, 11, 12, 13, 14, 15, 16, 17, 18) or on X chromosomal *M* factor, X^M, (11, 20). In addition to standard strains in which sex is determined by XX–XY heterosomal systems, a rapid invasion of autosomal sex determining factors in housefly populations in different parts of the world has been reported (12, 14, 19, 20, 21, 22, 23). In these populations, females and males both have two X chromosomes, but in the male, one of the autosomes carries the *M* factor. Marker genes are available on all of the autosomes, but not on the sex chromosomes. Thus sex–linked inheritance does not commonly occur. Either X or Y is needed for viability and fertility. *M* was found on all chromosomes, which was interpreted as *M* being part of a mobile element (24). In these populations, some female flies have a feminizing factor, *F,* of which one copy is epistatic to apparently any number of Y's and *M* factors (14, 25). Several dipteran species such as *Megaselia scalaris* (26), *Chironomus oppositus* and *Chironomus australis* (27, 28), do not have heteromorphic chromosomes but carry a dominant *M* factor.

The *M* factor has been demonstrated in the second, the third and the fourth linkage groups in the housefly populations of Turkey, and some individuals which were heterozygous and homozygous for *M* factors have also been identified (10). The results of *M* homozygosity and strong deviation from a 1:1 sex ratio indicated that *F* dominant factor and other *M* factors must have been in some of the Turkish housefly population. The objective of this study was to reexamine these populations for sex–determining factors.

Materials and Methods

Animals and genotypes

a) Wild type strains. Population cages were initiated with about 100 flies (males+females) collected in July 1995 from three different localities (Ordu, Giresun and Trabzon) in Turkey. In the first generation, the sex ratios of these three populations were recorded (Table 1). During the collection, flies were caught from different parts of each locality to build up a representative sample from the gene pool of each population.

b) Mutant strain I: All flies have *Ali–curve (ac)* wing shape mutation on the first, *aristapedia (ar)* bristles mutation on the second, *brown body (bwb)* color mutation on the third and *yellow eye (ye)* color mutation on the fourth and *snip wing (sn)* mutation on the fifth chromosome in homozygous condition. These are all recessive characters and distinguishable in homozygous condition. This strain also bears the standard XY–type sex determining mechanism.

c) Mutant strain II: Male flies have male–determining *(M)* factor, *brown body (bwb)* color mutation and *green eye (ge)* color mutation on the third chromosome in homozygous condition. Like *bwb, ge* mutation has recessive character and is distinguishable in homozygous condition. On the fourth chromosome, there is a dominant, *bald abdomen (Ba)* mutation, which is lethal is homozygous condition. Like females, males have XX chromosomes due to autosomal male determining *(M^{III})* mechanism.

d) Mutant strain III: Male flies have the same genetic constitution as the male flies of mutant strain II except the *bald abdomen* marker on the fourth chromosomes.

e) Standard strain: This is a standard wild type laboratory strain having a standard sex determining mechanism with XX females, XY males.

Laboratory conditions

All experiments were done at constant illumination (60 watt fluorescent lamp), 25±3°C and 70% relative humidity. Flies were reared as described by Hilfiker–Kleiner et al. (3).

Chromosomal location of *M* factor

To find out the chromosomal location of M factor(s), linkage analysis was carried out by using multimarker strain (mutant strain I). Techniques and procedures for analysing sex determinants in field strains have been described in detail elsewhere (11, 20). Single–pair crosses between field and standard strains provide more detail on the genetic composition of individuals. Linkage relationships of autosomal factors were determined by test–crossing F_1 (multimarked female x field male) males back to multimarked females in single pair and examining test–cross progeny for sex–limited segregation of visible markers. From each field strain (Ordu, Giresun and Trabzon), about 25 single–pair crosses were set up between wild type males and multimarked females.

Chromosomal location of *F* dominant factor

A serial crosses (Figure 1) were done for Ordu, Giresun and Trabzon populations to determine the chromosomal location of F–dominant factor and to keep it in a new continuous stock.

Virgin females from one of the wild populations were crossed with males from mutant strain II. For single pair crosses, 30 F_1 virgin females were crossed with males from mutant strain III. All of the males had *bald abdomen*, while all females had normal abdomens in the F_2 generation due to *F*–dominant factor on the fourth chromosome. Virgin females of these F_2 generations were taken to continue the crosses for purification of *F*–dominant factor in a cage. Single–pair crosses were done between these females and males from mutant strain II. Male and virgin female progenies of each single–pair cross were seperated from each other. Genotypes of the males in this third generation were studied by crossing them with virgin females from standard strain if all progenies were male. These results were possible if F_3 progenies were homozygous for *M* factor on the third chromosome (*M* in circle in figure 1 shows the *M* factor which might come from the wild population). Then the virgin sisters of the males which yielded only male progenies were crossed with males from mutant strain II, and all F_4 progenies of these single–pair crosses were put in a cage as a new stock having F–dominant factor on the fourth chromosome.

Results

Table 1 shows sex ratios in three populations during the first generation after collection. There was a deviation in favour of femaleness in these populations.

Sex–limited inheritance for *ac, ar, bwb, ye,* and *snp* genes were investigated in three field populations. Test–cross progenies of Giresun and Ordu populations showed sex–limited segragation for the *bwb* gene, indicating that some males in these populations carry an *M* factor on the third chromosome (M^{III}), as previously determined for some Turkish housefly populations (10). In addition, the existence of *M* factor on the fifth chromosome (M^{V}) by sex–limited inheritance of the *snp* gene was found in the Trabzon population.

Table 1. Sex ratios in Trabzon, Ordu And Giresun housefly populations during the first generation after collection.

Field strains	Male	Female	X^2
Trabzon	40.6 (%)	59.4 (%)	9.72
Ordu	43 (%)	55 (%)	7.02
Giresun	43 (%)	57 (%)	1.52
Total	43.7 (%)	56.3 (%)	16.76

Serial crosses (Figure 1) using female flies from Trabzon, Ordu and Giresun populations and males from some laboratory strains showed that the frequency of females having *F* dominant factor (F^{D}) on the fourth chromosome was high in these populations.

As can be seen from figure 1, only 30 F_1 females were chosen from each population for single–pair crosses. Mass cross was applied for the other F_1 females to find the location of *F*–dominant factor on the fourth chromosome. Recombination frequency between the *Ba* gene and *F*–dominant factor was found (Table 2) by examination of 188.471 flies for any recombination (normal males, *bald females*) between these two genes. The frequency of recombinants was 1.4638% indicating–the side–by–side location of these genes. In addition, all these F_1 populations showed deviations (Table 2) from the 1:1 sex ratio in favour of femaleness.

Discussion and Conclusion

Among dipteran insects, a variety of mechanisms for the genetic control of sex determination has evolved. Different sex–determining mechanisms have been described in the housefly. Standard strains have an XX–XY mechanisms, the Y chromosome being always associated with maleness (29). Non–standard strains (females and males XX) have autosomal dominant sex factors either for maleness (*M*) or for femaleness (*F*). *M* factors have now been observed on all chromosomes of the housefly karyotypes (10, 11, 12, 13, 18, 20). The females carried a fourth chromosomal *F* factor, which is epistatic to a number of *M* factors (14, 15). This phenomenon, referred to as polymorphism, has also been reported in Italy (12), in the British Isles (11, 20), in Japan (17, 23) and in Turkey (22). The high frequency of XX males and the presence of M^{II}, M^{III} and M^{IV} factor was found in some housefly populations in Turkey (10). As reported by Inoue et al. (13), individuals which were heterozygous for more than one *M* factor or homozygous for *M* factors were also identified in these populations. Some crosses between males from field populations x females from multimarked laboratory strains produced all male progeny (10) as reported by Mc Donald (15) and Denholm et al. (11, 21). Identification of *M* factor polymorphism and homozygosity indicates that there might be the other *M* factors and also *F* factor in these housefly populations in Turkey. This estimate has been confirmed by isolation of two new sex–determining factors (M^{V} and F^{D}) from the populations which were built up by recollected flies.

The evolution of non–standard sex determination mechanisms in houseflies appears to be a recent phenomenon. An invasion of these new factors (*M* and *F*) to the field populations has been reported in housefly populations in Europe (11, 12) and in Japan (17, 23). There are no comparable data, but the high frequency of XX males (22) and polymorphism of M factors (10) imply a reshaping of views regarding the sex determination

Table 2. Number of male flies (Ba^{+}) and female flies (*Ba*) having recombination between *Ba* and *F* dominant factor on the fourth chromosome.

Field strains	Male number	recombinants	Female number	recombinants	TOTAL number	recombinants
Trabzon	2583	9 Ba^{+}	4168	4 *Ba*	6751	13
Ordu	2515	6 Ba^{+}	3614	4 *Ba*	6129	10
Giresun	2231	3 Ba^{+}	3333	1 *Ba*	5564	4
Total	7329	18	11115	9	188471	(1.4639 %)

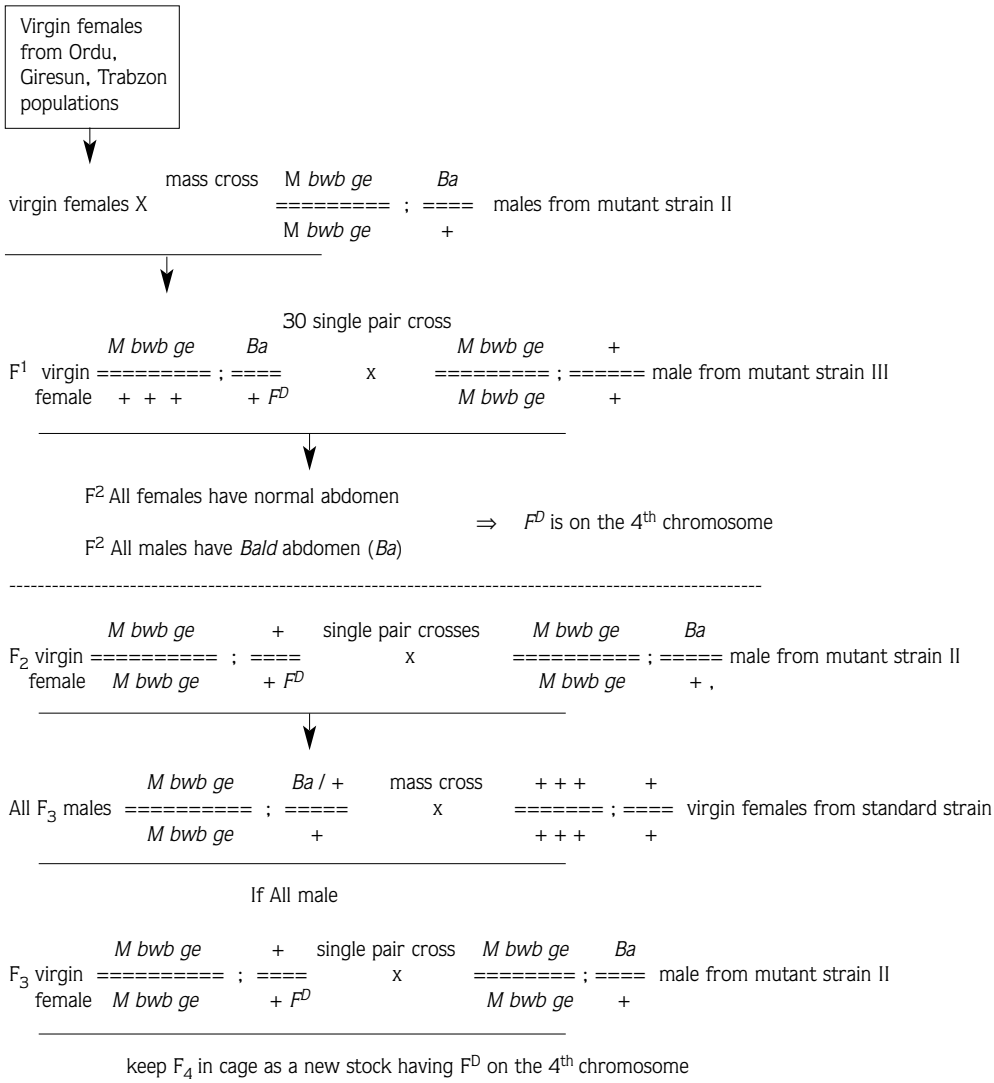

Figure 1. Crosses between females from field strains (Trabzon, Ordu, Giresun) and males from some laboratory strains to map F dominant factor.

mechanisms in natural populations of houseflies in Turkey, as seen in Europe and Japan.

There are some suggestions for the reason why non–standard genotypes have been favoured by selection in recent years. Transposition of *M* factors mediated by a transposable element or elements has been suggested as a cause of *M* factor polymorphism by Green (24). Both climate and the widespread use of insecticides have probably played a considerable role in the micro–evolution of sex–determinants in housefly populations (6, 11, 12, 25, 30), but the way this occurs is not known. These hypotheses, however, do not satisfactorily explain this invasion, and very detailed laboratory and field work will be required to perceive the cause(s) of this micro–evolutionary change in sex–determination mechanisms in housefly populations.

Acknowledgement

I am grateful to Prof. Dr. Andreas Dübendorfer for providing laboratory facilities for these experiments. I also thank Prof. Dr. Rolf Nöthiger, Monika Hediger, and Regula Schmidt for fruitful discussions and Raymond Grunder for technical assistance.

References

1. Hodgkin, J. (1992). Genetic sex determination mechanisms and evolution. Bio Essays, 14 (4): 253–261.
2. Nöthiger, R., and Steimann–Zwicky, M. (1985). A single principle for sex determination in insects. Cold Spring Harbour Symp. 50, 615–621.
3. Hilfiker–Kleiner, D., A. Dübendorfer, A. Hilfiker and R. Nöthiger (1994). Genetic control of sex determination in the ferm line and some of the houselfy, Musca domestica Development, 120: 2531–2538.
4. Hiroyoshi, T. (1977). Some new mutants and revised Linkage maps of the housefly, Musca domestica L. Japanese J. of Genetics, 52: 275–288.
5. Milani, R. (1967). The genetics of Musca domestica and other muscoid flies. In Genetics of Insect Vectors of Disease (ed. J. W. Wright and R. Pal) pp. 315–369. Amsterdam: Elsevier.
6. Hiroyoshi, T., Y. Fukumori, and H. Inoue (1982). Male crossing–over and location of the male determining factor on the third chromosome in a M^{III}–type strain of the housefly. Jpn. J. Genet. 57: 231–239.
7. Hoyes, R. F. (1965). Some new mutants of the housefly, Musca domestica. L. with notations of Related Phenomena. J. Econ. Entomol. 59: 133–137.
8. Perje, A.M. (1948). Studies on the spermatogenesis in Musca domestica L. Hereditas, 34: 209–232.
9. Stevens, N.M. (1908). A study of the germ cells of certain Diptera, with reference to the heterochromosomes and phenomena of synapsis, The J. of experimental Zoology 5: 359–374.
10. Çakır, Ş., R. Nöthiger and A. Kence (1998). Polymorphism of M factors in populations of the housefly. Musca domestica L. in Turkey (in press).
11. Denholm, I., M.G. Franco, P.G. Rubini, and M. Vecchi. (19857. Geographical variation in housefly (Musca domestica L.) sex determinants within the British Isles. Genet. Res. 47: 127.
12. Franco, M.N., P.G. Rubini and M. Vecchi (1982). Sex determinants and their distribution in various populations of Musca domestica L., of Western Europe. Genet. Res. 40% 279.
13. Inoue, H., Y. Fukumori, and T. Hiroyoshi (1983). Mapping of autosomal male–determining factors of the housefly. Musca domestica L. by means of sex reversal. Jpn. J. Genet. 58: 451–461.
14. Inoue, H., and T. Hiroyoshi (1982). A male–determining factor on autosome 1 and occurence of male recombination in the housefly, Musca domestica L. Jpn. J. Genet. 57: 221–229.
15. Mc. Donald, I.C., P. Evenson, C.A. Nickel, and O.A. Johnson, (1978). Housefly genetics: Isolation of female determining factor on chromosome 4. *Ann. Entomol. Soc. Am.* 71: 692–694.
16. Wagoner, D.E. (1969). Presence of male determining factors found on three autosomes in the housefly. Musca domestica L. Nature, 223: 187–188.
17. Hiroyoshi T. and Y. Fukumori. (1978). On the sex determination in wild populations of the housefly. Jpn. J. Genet. 53: 420–421.
18. Dübendorfer, A.D. Hilfiker–Kleiner, and R. Nöthiger (1992). Sex determination mechanisms in dipteran insects: the case of Musca domestica L. Developmental Biology. 3: 349–356.
19. Tomita, T. and Y. Wada. (1989). Multifactorial sex determination in natural populations of the housefly (Musca domestica L.) in Japan. Jpn. J. Genet. 64: 373–382.
20. Denholm, I., M.G. Franco, P.G. Rubini, and M. Vecchi, 1983. Identification of a male determinant on the X chromosome of housefly (Musca domestica L.) populations in South–East England. Genet. Res. 42, 311–322.
21. Denholm, I., P.G. Rubini, C. Rovati and M. Vecchi. (1990). Genetic basis of sex determination in two South African strains of housefly (Musca domestica L.) South African Journal of Science, 86: 41–42.
22. Çakır, Ş. and A. Kence. (1996). The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of Turkey. Genetica, 98: 205–210.
23. Rubini, P.G. and Franco, M.G. (1972). Localization of the male determining factor M present in strain of Musca domestica L. Genetica Agraria, 26: 217–232.
24. Green, M.M. (1980). Transposable elements in Drosophila and other diptera. Ann. Rev. Genet. 14: 109–120.
25. Bull, J.J. (1983). Evolution of sex determining mechanisms. pp. 30–32. Menlo Park. California: Benjamin/Cummings.
26. Mainx, F. (1964). The genetics of MegaSelra scalaris Loew (Phoridae): A new type of sex determination in Diptera. Am. Nat., 98: 415–430.
27. Martin, J., C. Kuvangkadilok. D.H. Peart, and B.T.O. Lee. (1980). Multiple sex determining regions in a group of related Chironomus species (Diptera: Chironomidae). Heredity, 44: 367–382.
28. Martin, J. and B.T.O. Lee. (1984). A phylogenetic study of sex determiner location in a group of Australian Chironomus Species Chromosoma 90: 190–197.
29. Boyes, J.W. and M.J. Corey. (1964). Somatic chromosomes of higher Diptera (IX Karyotypes of some Muscid Species). Canadian J. of Zoology, 42: 1015–1037.
30. Kence, M., and A. Kence. (1992). Genetic consequences of linkage between malathion resistance and an autosomal male determining factor in housefly (Diptera: Muscidae). J. Econ: Entomol. 85 (5): 1566–1570.