A High Frequency of Male Determining Factors in Male *Musca domestica* (Diptera: Muscidae) from Ipswich, Australia

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J. Med. Entomol. 46(1): 169–172 (2009)

ABSTRACT The male sex determining factor (M) in the house fly is linked to the Y chromosome in the ancestral condition, but can also be linked to another chromosome (I-V or X). However, descriptions of the linkage and frequency of M factors in different populations throughout the world are vastly incomplete. We collected house flies from a dairy in Ipswich, Australia, and determined that M was linked to chromosomes II, III, IV, and Y. Most males (69.8%) were homozygous for M on autosome II and/or III, and 92.3% of the males had multiple M factors. In all, there were 13 different male genotypes found. The high frequency of M, the presence of M on four different linkage groups, and the large number of male genotypes found in this population make it unique relative to other populations of house flies that have been examined.

KEY WORDS M factor, sex determination, linkage analysis, Insecta

The house fly, *Musca domestica*, is a serious threat to human and animal health. House flies are vectors of >100 human and animal intestinal diseases (Scott and Lettig 1962, Greenberg 1965, Keiding 1986). They are capable of transmitting parasites that cause diseases such as typhoid fever, cholera, bacillary dysentery, infantile diarrhea, tuberculosis, plague, leprosy, yaws, samonellosis, and anthrax (West 1951). Flies also transmit eye diseases such as trachoma and epidemic conjunctivitis (Keiding 1986). There are six million cases of childhood blindness each year caused by trachoma transmitted by flies (World Health Organization 2004). House flies were implicated in transmitting the deadly strain of *E. coli* that resulted in 11 deaths in Japan in 1996 (Iwasa et al. 1999). This is of concern with the proximity of humans to sources of high fly densities (such as dairies).

The male sex determination factor (M) in the house fly is linked to the Y chromosome in the ancestral condition, but can also be found linked to an autosome (I-V), or even rarely to the X chromosome (there are five autosome pairs, $2n = 12$). Autosomal males (A^M) have been found in six countries including the United States (Hiroyoshi 1964, McDonald et al. 1975, Shono and Scott 2003, Hamm et al. 2005), Japan (Hiroyoshi 1964, Hiroyoshi et al. 1982, Tomita and Wada 1989a, Shono and Scott 1990), Turkey (Cakir 1999), Italy (Franco et al. 1982), British Isles (Denholm et al. 1985), and South Africa (Denholm et al. 1990). However, populations consisting of males with M linked to multiple autosomes are relatively rare. Only one study, conducted in Japan, has found populations where individual males had M on more than two autosomes (Tomita and Wada 1989a). In that study, M was linked to four autosomes in 2 of 19 collections, was linked to all five autosomes in one location (Aio), and was linked to two or three autosomes in most of the other populations. Individual genotypes were reported for one population (Yumenoshima), where 68% of the males had multiple M factors and there were seven male determining genotypes present. Understanding the linkage and frequency of M factors in different populations from other parts of the world will provide valuable information about the evolution of this sex determining system in house flies.

Populations that contain individual males with multiple M factors (e.g., $III^M/III + Y^M$) or males homozygous for an A^M factor also contain females with F^D (McDonald et al. 1978, Tomita and Wada 1989a, b) to keep the production of offspring near a 1:1 (male: female) ratio. F^D is epistatic to M, produces females in the presence of up to three copies of M (Dübendorfer and Hediger 1998), and is located on the fourth chromosome (McDonald et al. 1978, Cakir 1999).

An understanding of the factors underlying the relative frequency of Y^M and A^M, as well as the identification of M, may offer new insights into fly reproduction that could lead to new control methods, such as the release of homozygous M males or transgenic males (carrying multiple copies of M, or having high levels of expression of M) into closed systems, such as poultry facilities. This would lead the following generation to produce all males and thus provide control. Additional strategies will follow as a deeper understanding of this biological system is attained.

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0022-2585/09/0169–0172$04.00/0

This study determined the frequency and genetic linkage of M in a field collected house fly population from Ipswich, Australia. The genotypes of individual males were also determined. This is the first report on the linkage of M from an Australian field population and only the second from the southern hemisphere. Our results indicate this population is the most extreme thus far reported; both in terms of the high frequency of M and the large number of male genotypes present.

Materials and Methods

Adult house flies were collected by sweep netting within a dairy milk barn in mid-January 2007 from Ipswich, Australia. First generation pupae were shipped to the United States (USDA permit 101208) and reared as described previously (Hamm et al. 2005). Males from the second-generation laboratory population were used as described below. A Y^M laboratory strain, aabys (Hamm et al. 2005), with visible recessive markers *ali-curve, aristapedia, brown body, yellow eyes,* and *snip wings* on autosomes I, II, III, IV, and V, respectively, was used for linkage analysis.

To determine the linkage of M, a backcross experiment was carried out as previously described (Hamm et al. 2005). Briefly, Ipswich second-generation male flies ($n = 110$) were individually crossed to four to six unmated aabys females. Emerging F_1 progeny males and females were counted. Three to eight F_1 sons from each original male were individually used in a backcross with four to six aabys females as described above. Eight males were crossed if the F_1 generation produced exclusively males. The emerging backcross individuals were phenotyped according to sex and markers to determine the linkage of M. For example, III^M males were identified if all backcross females were *bwb/bwb* (brown body) and all males were wild type (*bwb*/+).

Linkage of a mutant marker with sex in the backcross, as well as sex ratios (of the F_1 and backcross) were used in determining the parental male genotype. If only males were produced in the F_1, this indicated that the parental male was homozygous M for at least one chromosome. Backcross phenotypes showed which chromosome(s) were carrying the M factor in individual sons. When F_1 and backcross phenotypes alone could not distinguish between genotypes, backcross ratios of females to males (1:1, 1:3, 1:7, 1:15, and 1:31) were tested. A χ^2 test was conducted on the observed backcross sex ratios to determine whether they fit the expected ratios given by specific genotypes. Data were not included if they did not fit one of the ratios and could not be determined by F_1 sex ratio and backcross phenotype. None of the data fit more than one predicted χ^2 ratio, consistent with unambiguous assignment of linkage for each male.

Results and Discussion

Only male progeny were produced in 69.8% of the F_1s, indicating a high number of males were homozygous M for at least one autosome. Backcrosses showed that 92.3% of the males had multiple M factors (Table 1). The Ipswich population contained 13 different male genotypes (Table 1). Presence of males that are homozygous for M and/or which have M on multiple chromosomes would skew the sex ratio, producing 3–15 times more males than females, in the absence of F. This strongly suggests F^D must be present in this population (no noticeable skew of sex ratio was seen in the original Ipswich population). We are unable to distinguish Y^M from X^M in our tests because of the lack of genetic markers on the sex chromosomes of house flies. Therefore, we used Y^M to designate Y^M or X^M males. Males with Y^M were relatively rare (15.4%) and were found only in combination with A^M. The frequency of III^M, II^M, Y^M, and IV^M was 0.7, 0.44, 0.07, and 0.02, respectively. There were more individuals than expected (based on the frequencies above) for the III^M/III^M, II^M/II^M, II^M/II III^M/III^M IV^M/IV XY^M, and III^M/III IV^M/IV XY^M genotypes (Table 1).

Table 1. Observed and expected male genotypes in house flies collected from a dairy in Ipswich, Australia, in 2007

Genotype				Observed	Expected	χ^2	P
II / II	III^M / III^M	IV / IV	X / X	11	5.68	4.994	0.0254
II^M / II	III^M / III	IV / IV	X / X	8	7.73	0.009	0.9244
II^M / II	III^M / III^M	IV / IV	X / X	5	9.02	1.793	0.1806
II^M / II^M	III^M / III^M	IV / IV	X / X	5	3.48	0.665	0.4148
II^M / II^M	III / III	IV / IV	X / X	4	0.64	17.68	<0.0001
II / II	III^M / III	IV / IV	X / X	3	4.87	0.715	0.3978
II^M / II^M	III^M / III	IV / IV	X / X	2	2.98	0.322	0.5704
II^M / II	III^M / III^M	IV / IV	Y^M / X	2	1.36	0.304	0.5817
II^M / II	III / III	IV / IV	X / X	1	1.66	0.261	0.6094
II / II	III^M / III	IV / IV	Y^M / X	1	0.73	0.098	0.7547
II^M / II	III^M / III^M	IV^M / IV	Y^M / X	1	0.06	16.24	<0.0001
II^M / II	III / III	IV / IV	Y^M / X	1	0.25	2.256	0.1331
II / II	III^M / III	IV^M / IV	Y^M / X	1	0.03	31.48	<0.0001

In addition to the genotypes listed above, there were five males that were $II^M/II^M;III^M/III$ or $II^M/II;III^M/III^M$ or $II^M/$ $II^M;III^M/III^M$ and two males that were $II^M/II^M;III^M/III;Y^M/X$ or $II^M/II;III^M/III^M;Y^M/X$.

The Ipswich house fly population is unique from those previously described because 69.8% of the males were A^M/A^M, the frequency of males with multiple M factors was very high, M was linked to four chromosomes, and at least 13 male genotypes were detected. The high frequency of homozygous M males and males with M on multiple autosomes implies that F^D exists at a high frequency in this population. F^D has been confirmed (D. Bopp, personal communication) in females from this strain, although no frequency was determined because the population underwent a severe population decline after being maintained in the laboratory for three generations.

Our results differ from a report using an Australian laboratory strain that was collected from Brisbane

(≈40–50 km from Ipswich) and held in the laboratory for an unstated period of time. This strain had II^M, III^M, and V^M males (Wagoner 1969). In contrast, we did not detect any V^M males but did find IV^M and Y^M. It is possible that the laboratory population had changed since it was colonized, or the frequency and linkage of M have changed in the field population between the late 1960s and 2007. Studies looking at the changes in frequency of A^M and Y^M males under field and laboratory conditions would help to explain how rapidly linkage frequencies can change and the effects of environment on the males with different linkages of M. Another Australian strain, collected at Kingsford (≈1970), was III^M (and XX) (Lester et al. 1979).

It seems that worldwide variation in linkage and frequency of M fluctuates greatly. The number of autosomes carrying M factors also varies. In Japan, populations have been found where M is linked on every autosome and the Y (Tomita and Wada 1989a). In contrast, linkage of M has only been reported on III and Y in the United States (Hamm et al. 2005). Populations with M on multiple chromosomes could theoretically arise from one of two different mechanisms. First, populations with M on different chromosomes could mix and, provided F^D was present, produce males with two M factors. Alternatively, M could arise on a second autosome in a population where it was already present on another chromosome. With the assumptions that M is the same factor on all autosomes, that autosomal males arose as a transposition of M from Y (Tomita and Wada 1989a, Schmidt et al. 1997) and that A^M strains usually lose the Y chromosome (Hiroyoshi 1964, Wagoner 1969, Franco et al. 1982, Denholm et al. 1983, Denholm et al. 1990) suggests that the first mechanism is the most likely. It is unclear what selective forces are responsible for some populations having a single M (Y^M or A^M) and a very low (or zero) frequency of F^D (i.e., males being the heterogametic sex), whereas other populations have males homozygous for M, contain M on multiple chromosomes, and have a high frequency of F^D (females being the heterogametic sex).

In conclusion, we found that the Ipswich population of house flies has the largest number of male genotypes ever reported. M is linked to chromosomes II, III, IV, and Y, often in combination and/or as homozygotes on autosomes II and/or III. It would be of interest to look at other Australian populations to determine the frequency and linkage of M and also determine whether there is a cline as reported in Japan (Tomita and Wada 1989a) and the United States (Hamm et al. 2005). It would also be of great value to examine the frequency and linkage of M (and frequency of F) in populations from South America, Central America, China, and other regions to get more detailed information about the global patterns of sex determination in house flies.

Acknowledgments

We thank B. Tozer, J. Deacutis, and J. Hogsette for assistance with collections and C. Leichter, T. Lazo, and G. Lin for technical assistance.

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Received 11 July 2008; accepted 27 September 2008.