A Cline in Frequency of Autosomal Males Is Not Associated with Insecticide Resistance in House Fly (Diptera: Muscidae)

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ABSTRACT Geographic variation in the chromosomal location of the male sex determining factor (M) was studied in four house fly, *Musca domestica* L., populations from the eastern United States. We found a strong clinal trend (29° 41′ latitude in Florida to 44° 2′ in Maine) in which the percentage of standard XY^M males increased with increasing latitude. In Florida, 100% of the males possessed the M factor on the third autosome (III^M). North Carolina had 20% III^M males and 2.35% with both Y^M and III^M. Fewer III^M males were found in New York (4.35%). Populations from Maine contained 100% XY^M males. In two of three standard laboratory-susceptible strains, all males carried M on an autosome ("autosomal males" or A^M): CS (III^M) and SRS (V^M). Insecticide bioassays of four field-collected strains led us to conclude that resistance is not correlated with sex determination over a broad range of insecticides. For example, high levels of resistance to permethrin (86–99% survival at a diagnostic concentration) were found in all four field-collected strains. The five other insecticides evaluated showed varying levels of resistance among field strains. We conclude that a cline is present in house fly populations from the eastern United States with 100% III^M males in the south and entirely Y^M males in the north and that insecticide resistance is not a key factor influencing the evolution or linkage of M.

KEY WORDS sex determination, evolution, crossover frequency, pyrethroid, organophosphate

IN HOUSE FLIES, *Musca domestica* L., sex is determined by a dominant factor, M, which is located on the Y chromosome in "standard" populations. Thus, males are XY^M and females are XX (Hiroyoshi 1964, Dübendorfer et al. 2002). This is believed to be the ancestral state of sex determination in house flies (Bull and Charnov 1977, Denholm et al. 1983). However, there are "autosomal male" (A^M) strains in which the M factor is located on one or more of the five autosomes (I–V) (Franco et al. 1982, Inoue et al. 1983, Tomita and Wada 1989) or even rarely on X (Schmidt et al. 1997). The M factor located on Y is the same as the M located on any of the other autosomes (Tomita and Wada 1989, Schmidt et al. 1997). In the A^M strains females are XX and males are also XX (or XO) (Hiroyoshi 1964, Wagoner 1969, Franco et al. 1982, Denholm et al. 1983, Denholm et al. 1990). III^M seems to be the most frequently occurring type of autosomal male, being found in field populations from the United States (McDonald et al. 1975), Japan (Hiroyoshi 1964), Turkey (Cakir and Kence 1996), South Africa (Denholm et al. 1990), and Europe (Franco et al. 1982; Denholm et al. 1983, 1985). However, the appearance of autosomal males is not random. Latitudinal and altitudinal clines existed for locations in western Europe from the south (Sicily) to the north (Denmark and Iceland) (Franco et al. 1982). A radial cline was described in the British Isles having a gradual increase in the number of XY^M males upon moving north, east, and west (Denholm et al. 1985). One study in Japan found only Y^M males in the four most northern collection sites, although there was no significant north-south cline (Tomita and Wada 1989). It seems that the frequency of A^M has been increasing in house fly populations (possibly coincident with the evolution of insecticide resistance) (Tomita and Wada 1989). In addition, autosome III has an important gene for resistance to pyrethroids and DDT (*kdr* and *super-kdr*) (Shono 1985) and is the most frequent location for an autosomal male factor (Franco et al. 1982, Tomita and Wada 1989). Thus, it has been suggested that autosomal males may be causally related to the evolution of insecticide resistance (Hiroyoshi 1980) or due to a consequence of tight linkage to resistance genes (Franco et al. 1982, Bull 1983, Kence and Kence 1992). However, no studies have examined both of these phenomena in field populations simultaneously.

In this study, we examined the linkage of M in four populations of house flies in the eastern United States and discovered the presence of a latitudinal cline. We also evaluated resistance to six insecticides to determine whether there was an association with the presence of A^M males.

Materials and Methods

House Fly Strains. Adult house flies were collected throughout summer 2002 from Florida (FL, Alachua

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County), North Carolina (NC, Wake County), New York (NY, Schuyler County), and Maine (ME, Androscoggin County). Adults were captured with a sweep net from inside dairy barns and around calf hutches, except for NC where pupae were collected from around calf hutches. Field-collected animals were used to establish laboratory colonies.

Three insecticide susceptible laboratory strains were used: 1) Cornell susceptible (CS, created by crossing the susceptible S+ strain (originally from Dr. F. W. Plapp, Jr., Texas A&M University, College Station, TX) with another susceptible strain (flies collected in NY crossed to an inbred strain from G. Georghiou, University of California, Riverside, CA and then crossed to a strain from the USDA in Beltsville, MD; Pimentel and Burgess 1985) in 1992 (Scott et al. 1996); 2) SRS (created in 1961 from a cross between an Italian inbred strain from Pavia and a strain from the University of Wageningen, Wageningen, The Netherlands; Keiding 1999) was obtained from S. Kasai (Tokyo, Japan); and 3) *aabys*, a strain with visible recessive markers *ali-curve*, *aristapedia*, *brown body*, *yellow eyes*, and *snip wings* on autosomes I, II, III, IV, and V, respectively. All colonies were reared as described previously (Scott et al. 2000).

Linkage Analysis. One field-collected (or first generation in FL) male was crossed to four unmated *aabys* females. Virgin females were collected within 8 h of emergence. One hundred males were crossed per site, and this resulted in an average of 77 crosses with flies for the subsequent backcross analysis (see below). Flies were kept in 270-ml paper hot cups (International Paper, Post Turbhe, Navi Mumbai) with poly chiffon tops and were fed granulated sugar:powdered milk (1:1) for 3 d. Water was provided using saturated cotton. After 3 d, flies were placed into cups with media (Scott et al. 2000) to oviposit. During this time, flies were provided with cotton soaked in a 10% sugar water solution. Media cups were changed every other day for 7–10 d. Media cups with eggs were stirred and additional medium was provided the day adult flies were removed. Cups were then misted every day with distilled water for 4 d.

Emerging F_1 males and females were counted. Three F_1 males from each original male were individually used in a backcross with four *aabys* females as described above. If the F_1 had >70% males, six to 10 backcrosses were made. The emerging backcross individuals were phenotyped according to sex and markers. Autosomal males were identified by having all (>99%) female (and <1% male) progeny associated with a specific marker. For example, if *brown body* (*bwb*, on autosome III) females are found in the backcross, but no *bwb* males are found, we would conclude that M is linked to autosome III. Similarly, Y^M males were identified by the lack of association between sex and any of the autosomal markers.

Theoretical Considerations. Male house flies have the ability to produce a variety of sex ratios, depending on their genetic makeup (Fig. 1). Males that contain only one heterozygous form of the M factor will produce a 1:1 ratio of males to females. This 1:1 ratio can be found if the male is Y^M or A^M. If only males are produced in a cross with *aabys*, we conclude that the male is homozygous for one M factor. This male may or may not contain additional M factors on another autosome. A male carrying two heterozygous forms of the M factor would produce a 3:1 ratio of males to females. A 7:1 male to female ratio is produced when three M factors exist in heterozygous form. These situations all assume that the female does not carry the F factor, which is epistatic to M and is found in populations that contain individuals homozygous for M. F is assumed not to exist in the populations we examined, because there were no males homozygous for an autosomal male factor.

	Male Genotype	F_1 Sex Ratio (Male:Female)
A)	$\frac{III^M}{III}$ or Y^M	1:1
B)	$\frac{III^M}{III^M}$ or $\frac{II^M}{II}$; $\frac{III^M}{III^M}$	1:0
C)	$\frac{II^M}{II}$; $\frac{III^M}{III}$	3:1
D)	$\frac{II^M}{II}$; $\frac{III^M}{III}$; $\frac{IV^M}{IV}$	7:1

Fig. 1. Predicted sex ratios of offspring produced by standard females and males having M factors in four possible configurations (A–D). II^M, III^M, and IV^M are used to represent the heterozygous or homozygous condition. Any of the five autosomes could exhibit these genotypes.

Chemicals. Permethrin (99.7%, *cis:trans* 45:55) was from Syngenta (Greensboro, NC). Cyfluthrin (*cis:trans* 40:60) was from Bayer (Kansas City, MO). Pyrethrins (51.28%) were from MGK Co. (Minneapolis, MN). Tetrachlorvinphos (99.5%) was from Chem Service (West Chester, PA). Dimethoate (99.7%) was from BASF Corp. (Mount Olive, NJ). Methomyl (99.8%) was from DuPont (Wilmington, DE). Piperonyl butoxide (PBO, 90%) was from Aldrich (Milwaukee, WI).

Bioassays. Six insecticides were evaluated by a residual contact bioassay (except methomyl) at the diagnostic concentrations used for resistance monitoring (Kaufman et al. 2001). Glass jars (230 ml, internal surface area is 180 cm^2) were treated with 1 ml of insecticide (in acetone), the acetone was allowed to evaporate for at least 30 min, and then 25 3–6-d-old females were placed inside. For pyrethrins plus PBO (these are used together in commercial formulations), 1 ml of each solution was applied to the jar. PBO was applied at a dose of 400 μg per jar. Two dental wicks (2.5 cm) soaked in 15% sugar water were provided in each jar. Methomyl is formulated as a bait and therefore required a feeding bioassay. Flies were exposed to methomyl by using two dental wicks that had been soaked in 15% sugar water with the desired concentration of methomyl and held in untreated glass jars (Kaufman et al. 2001). Each insecticide was tested on at least 150 house flies for all strains. Bioassays were

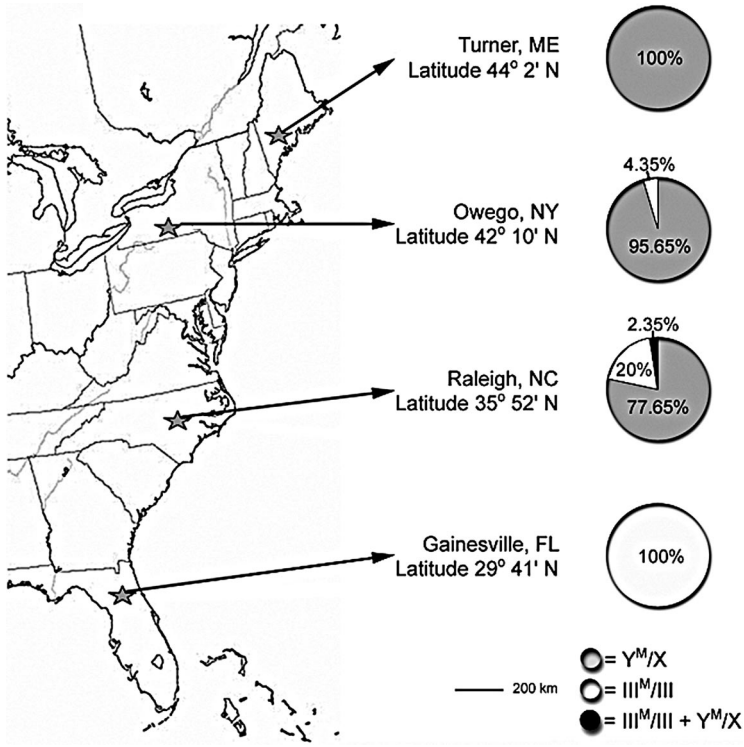

Fig. 2. Map of the eastern United States indicating the locations where house flies were collected. Pie charts indicate the relative frequency of III^M/III, Y^M/X, and $III^M/III;Y^M/X$ males.

kept at 25°C with a photoperiod of 12:12 (L:D) h. Water was added to dental wicks after 24 h, and mortality was assessed after 48 h. Flies were considered dead if they were unable to right themselves.

Results and Discussion

The linkage of M in house fly populations from the eastern United States reveals a significant cline (Fig. 2). All males from FL were III^M (identified by all backcross females having *bwb*, $n = 9910$). NC males were 77.65% Y^M, 20% III^M, and 2.35% had both III^M and Y^M ($n = 3425$). In NY, males were predominately Y^M with only 4.35% being III^M ($n = 477$). Males from ME were 100% Y^M ($n = 5715$). Thus, there is a latitudinal cline of Y^M and III^M males in the eastern United States, with higher percentages of autosomal males in the south and decreasing percentages upon moving north. This result is consistent with findings in Japan (Tomita and Wada 1989) and Europe (Franco et al. 1982) where a latitudinal cline was identified with autosomal males predominating in the south. Latitudes of the Japanese populations seem to range from ≈27° N in the south to ≈44° N in the north (Tomita and Wada 1989), whereas European latitudes were ≈37° in the south and 65° in the north. There is a similarity in the range of latitudes of our study (Fig. 2) and those in Japan.

Flies collected in 1973 from FL were shown to be 100% III^M (McDonald et al. 1975). This leads to the conclusion that there is some selective advantage for males to be III^M in FL because of its stability in the population for the past 30 yr. A^M house flies (III^M) were first reported in NY in 1998 (Shono and Scott 2003). Research on NY flies from 1980 (Scott et al. 1984) and 1987 (Konno and Scott 1991) did not show signs of autosomal males present in the population. This led to the suggestion that A^M is a recent phenomenon and might be increasing in the state. However, the nature of these three studies did not permit quantification of the frequencies of autosomal males. Autosomal males also have been described as a recent invasion in populations from Japan (Tomita and Wada 1989) and Italy (Franco et al. 1982) due to the increased frequencies found from the 1960s to the 1980s.

The linkage of M also was investigated in three laboratory susceptible strains. CS males were 100% III^M (55 males were used for the initial single male crosses, 54 backcrosses were established, and 6,587 flies from the backcross were phenotyped). SRS males were 100% V^M (i.e., all SRS backcross females had *snip wings*) with 945 flies phenotyped (55 males were used for the initial crosses of single males, and 17 backcrosses were established). This result is in contrast with previous reports (Milani et al. 1967, Franco et al. 1982) that SRS was Y^M, indicating the linkage of M in this strain changed between 1982 and 2003. Twenty-five *aabys* males were individually examined to determine the location of M in this strain. Four CS females were crossed per *aabys* male. Individually, three F_1

Table 1. Percentage of crossover events occurring between *bwb* and M in males of three field-collected strains (FL, NC, and NY) and two laboratory strains (CS and SRS) of house fly

Strain	% crossover	No. offspring scored	No. crossover events
FL	0.06	9910	6
NC	0.03	3425	1
NY	0	477	0
CS	0.08	6587	5
SRS	0.53	945	5

males from each of 14 lines were backcrossed to *aabys* females. The *aabys* strain was 100% Y^M with 1,604 flies phenotyped.

Crossover in male house flies seems to be very rare, but it has been quantified in one strain (Inoue et al. 1983). Given that *bwb* and M are ≈46 centimorgans apart on autosome III (Inoue et al. 1983), our experiments allowed us to quantify the frequency of crossover between *bwb* and M in males from five strains. The percentage of crossover in the FL, NC, NY, CS, and SRS strains is shown in Table 1. Crossover events occurred most frequently in the SRS strain (0.53%). The NY field-collected flies showed no crossover events, although this may be a function of the lower number of offspring scored in this population. The low frequency of crossover observed in males from these strains is consistent with a previous study (Inoue et al. 1983), but our results indicate the frequency of crossover is variable between strains.

Bioassay results showed great variation in the percentage of survival at diagnostic concentrations (i.e., a value that reflects the level of resistance) among the states, as well as insecticides, with the exception of permethrin (Fig. 3). Cyfluthrin resistance was variable with 35% survival in NC, 30% in ME, 28% in FL, and 18% in NY. Resistance to pyrethrins + PBO decreased in order of ME (61%) > NC (44% survival) ≈ FL (42%) > NY (18%). Resistance to methomyl was highest in ME (99% survival), followed by NY at 83%, NC at 70%, and FL at 14%. ME also had the highest resistance to dimethoate (83% survival), followed by NC (49%) and NY (48%). Flies from FL showed very little resistance to dimethoate (<1%). Tetrachlorvinphos resistance was highest in ME (50%), followed by NC (37% survival), NY (31%), and FL (11%). Permethrin was found to have high levels of resistance among all four states, ranging from 86% survival in NY to 99% in NC. These high levels of permethrin resistance are likely due to the extensive use of this insecticide in all four states since it was registered in 1985.

If the frequency of A^M is associated with insecticide resistance, we would expect higher levels of resistance in the FL strain and the lowest levels of resistance in the ME strain (to one or more insecticides). However, flies from FL, with 100% autosomal males, exhibited the lowest levels of resistance to three compounds and similar levels of resistance to the other three insecticides. House flies from ME were 100% Y^M and had high levels of resistance (>80% survival at the diagnostic concentration) to three insecticides. Thus, the bioassay data indicate that high resistance levels are not associated with the presence of autosomal males as was suggested previously (Franco et al. 1982, Kence and Kence 1992).

Due to the warmer climate in FL, there are more generations of flies produced, and more applications of insecticide are used, especially relative to NY and ME. Surprisingly, FL house flies did not have the highest levels of resistance (Fig. 3). Although the low levels of resistance to dimethoate and tetrachlorvinphos can be attributed to their low level of use in the state, the other four insecticides are all widely used (C. Geden, personal communication).

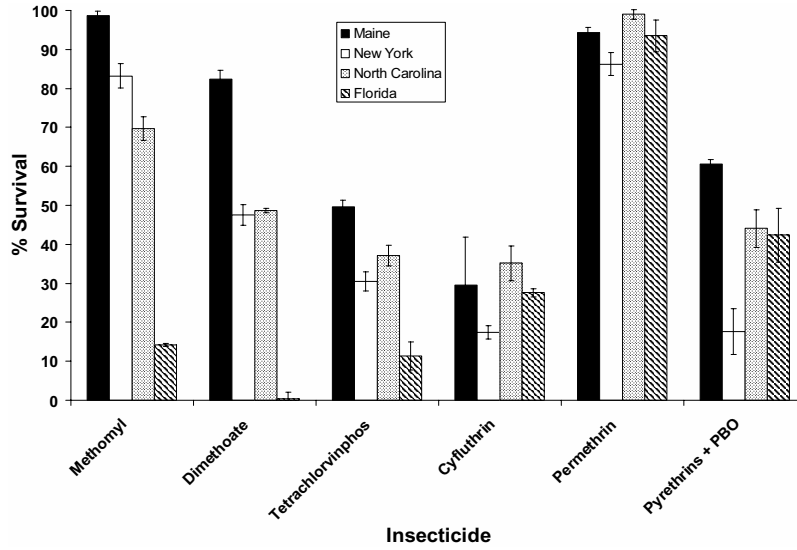

Fig. 3. Levels of resistance to six insecticides (scored as percentage of survival by using a diagnostic concentration) in house flies collected from four states in 2002. Bars represent the means (±SD).

Table 2. Monitoring of insecticide resistance in house flies from New York during 1987, 1999, and 2002

Insecticide	June 2002	June 1999	July and Aug. 1987
Permethrin	86	69	19
Cyfluthrin	18	31	
Pyrethrins + PBO	18	11	
Methomyl	83	51	
Dimethoate	48	8	80
Tetrachlorvinphos	31	31	

All values are percentage of survival at the diagnostic concentration. June 1999 data are from Kaufman et al. (1999) and July and August 1987 data are from Scott et al. (1989)

Percentage of survival in NY field-collected flies was documented in 1987 for permethrin (19%) and dimethoate (80%) (Scott et al. 1989). Flies from NY were examined again in 1999 for the same insecticides tested here (Kaufman et al. 2001). This provides a comparison for the changes in resistance over the 15-yr period for two insecticides and 3 yr for all others (Table 2). Resistance has increased in the state for permethrin, pyrethrins + PBO, and methomyl. Cyfluthrin resistance has decreased two-fold. Tetrachlorvinphos resistance has stayed the same. Flies were highly resistant to dimethoate in 1987 and had decreased to low levels by 1999. There seems to be a resurgence in recent years, but not reaching as high a level as was reported in 1987. These patterns are consistent with use patterns in the state (P. Kaufman, personal communication).

Two previous studies at NY dairies showed that resistance levels were similar for all insecticides regardless of the spray regime used at the site (Scott et al. 1989, Kaufman and Rutz 2001). We show that resistance levels vary greatly between states with the exception of permethrin, which has high levels in all states. One important consideration for the development of a resistance management program is to identify the size of the target area (single site, county, state, or region) over which the program must be implemented to maximize the chances of success (i.e., determine pest mobility). The previous studies of resistance in NY suggested house flies were very mobile, and resistance management programs would have to be carried out over a very large area. Our results show that patterns of insecticide use in different states produce unique patterns of insecticide resistance. Thus, it seems that for house fly control a successful resistance management program could be undertaken on a statewide scale with reasonable chances for success.

In conclusion, we determined that a latitudinal cline exists in eastern United States house flies with the linkage of III^M predominant in the south and absent in the north. Latitudinal clines have been revealed in three locations (United States, Europe, and Japan), two of which have collections made at similar latitudes (United States and Japan) (Franco et al. 1982, Tomita and Wada 1989). The linkage of III^M seems to have been fixed in the FL house fly populations since at least 1971 (McDonald et al. 1975). The only other population reported to have 100% III^M males was from Hachijo, Japan, although only 28 males were tested (Tomita and Wada 1989). House flies in FL must have some selective advantage to being III^M. It is unknown what advantage may be working to select for these individuals. Climatic conditions have been suggested to influence the linkage of A^M in both type and frequency (Franco et al. 1982). Although the development of insecticide resistance also has been suggested as a cause for autosomal males (Franco et al. 1982, Tomita and Wada 1989, Kence and Kence 1992), our data indicate no correlation between insecticide resistance and the frequency or linkage of M.

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References Cited

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin/Cummings, Menlo Park, CA.

Bull, J. J., and E. L. Charnov. 1977. Changes in the heterogametic mechanism of sex determination. Heredity 39: 1–14.

Cakir, S., and A. Kence. 1996. The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of turkey. Genetica 98: 205–210.

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.

Denholm, I., M. G. Franco, P. G. Rubini, and M. Vecchi. 1985. Geographical variation in house-fly (*Musca domestica* L.) sex determinants within the British Isles. Genet. Res. 47: 19–27.

Denholm, I., P. G. Rubini, C. Rovati, and M. Vecchi. 1990. Genetic basis of sex determination in two South African strains of house fly. S. Afr. J. Sci. 86: 41–43.

Dübendorfer, A., M. Hediger, G. Burghardt, and D. Bopp. 2002. *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. Int. J. Dev. Biol. 46: 75–79.

Franco, M. G., 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: 279–293.

Hiroyoshi, T. 1964. Sex-limited inheritance and abnormal sex ratio in strains of the housefly. Genetics 50: 373–385.

Hiroyoshi, T. 1980. Formal genetics of the housefly in relation to insecticide resistance. *In* Proceedings of the 16th International Congress of Entomology, Kyoto, Japan.

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.

Kaufman, P. E., and D. A. Rutz. 2001. Susceptibility of house flies (Diptera: Muscidae) exposed to commercial insecticides on painted and unpainted plywood panels. Pest Manage. Sci. 58: 174–178.

Kaufman, P. E., J. G. Scott, and D. A. Rutz. 2001. Monitoring insecticide resistance in house flies (Diptera: Muscidae) from New York dairies. Pest Manage. Sci. 57: 514–521.

Keiding, J. 1999. Review of the global status and recent development of insecticide resistance in field populations of the housefly, *Musca domestica* (Diptera: Muscidae). Bull. Entomol. Res. 89: S7–S67.

Kence, M., and A. Kence. 1992. Genetic consequences of linkage between malathion resistance and an autosomal male-determining factor in house fly (Diptera: Muscidae). J. Econ. Entomol. 85: 1566–1570.

Konno, Y., and J. G. Scott. 1991. Biochemistry and genetics of abamectin resistance in the house fly. Pestic. Biochem. Physiol. 41: 21–28.

McDonald, I. C., D. E. Overland, R. A. Leopold, M. E. Degrugillier, P. B. Morgan, and H. C. Hofmann. 1975. Genetics of house flies: variability studies with North Dakota, Texas, and Florida populations. J. Hered. 66: 137–140.

Milani, R., P. G. Rubini, and M. G. Franco. 1967. Sex-determination in the housefly. Genet. Agaria 21: 385–411.

Pimentel, D., and M. Burgess. 1985. Effects of single versus combinations of insecticides on the development of resistance. Environ. Entomol. 14: 582–589.

Schmidt, R., M. Hediger, S. Roth, R. Nothiger, and A. Dübendorfer. 1997. The *Y*-chromosomal and autosomal male-determining *M* factors of *Musca domestica* are equivalent. Genetics 147: 271–280.

Scott, J. G., T. Shono, and G. P. Georghiou. 1984. Genetic analysis of permethrin resistance in the house fly, *Musca domestica* L. Experientia 40: 1416–1418.

Scott, J. G., R. T. Roush, and D. A. Rutz. 1989. Insecticide resistance of house flies from New York dairies (Diptera: Muscidae). J. Agric. Entomol. 6: 53–64.

Scott, J. G., P. Sridhar, and N. Liu. 1996. Adult specific expression and induction of cytochrome $P450_{lpr}$ in house flies. Arch. Insect Biochem. Physiol. 31: 313–323.

Scott, J. G., T. G. Alefantis, P. E. Kaufman, and D. A. Rutz. 2000. Insecticide resistance in house flies from caged-layer poultry facilities. Pest. Manage. Sci. 56: 147–153.

Shono, T. 1985. Pyrethroid resistance: importance of the *kdr*-type mechanism. J. Pestic. Sci. 10: 141–146.

Shono, T., and J. G. Scott. 2003. Spinosad resistance in the house fly, *Musca domestica*, is due to a recessive factor on autosome 1. Pestic. Biochem. Physiol. 75: 1–7.

Tomita, T., and Y. Wada. 1989. Multifactorial sex determination in natural populations of the housefly (*Musca domestica*) in Japan. Jpn. J. Genet. 64: 373–382.

Wagoner, D. E. 1969. Presence of male determining factors found on three autosomes in the house fly, *Musca domestica.* Nature (Lond.) 223: 187–188.

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