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Changes in the frequency of Y^M versus III^M in the housefly, *Musca domestica* L., under field and laboratory conditions

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Summary

In the housefly, *Musca domestica* L., sex is usually determined by a dominant factor, M, located on the Y chromosome. However, there are 'autosomal male' (A^M) populations in which the M factor is located on one or more of the five autosomes (I–V) or on X. We examined changes in the frequency of A^M and Y^M males in North Carolina populations of houseflies after 4 years in the laboratory (NC Lab 02:06) and after 4 or 5 years in the field (NC 2006 and NC 2007). In 2002, 77·7% of the male houseflies were III/III;XY^M, 20% were III^M/III;XX, and 2·3% were III^M/III;XY^M. After 4 years in the laboratory, III^M/III males disappeared and 17·4% of the males were X^MY^M. Conversely, 4 years later, the field population was relatively unchanged from 2002. Thus, there was a strong selection against III^M/III males in the laboratory, but not in the field. Field-collected flies from 2007 indicated a slight increase in the frequency of XY^M males and a slight decrease in the frequency of III^M/III males (relative to 2002 and 2006), suggesting that the relative frequency of XY^M and III^M/III can vary slightly over time in field populations. The detection of X^MY^M males in 2007 offered the opportunity to evaluate the frequency of the female-determining F^D factor, which was found to be present in both the laboratory and field populations, but frequencies varied greatly. The present study represents the first report of F^D in houseflies from North America. The significance of these results, relative to observed clines in A^M versus Y^M males, is discussed.

1. Introduction

In the housefly, *Musca domestica* L., sex is determined by a dominant factor, M, located on the Y chromosome. There appears to be multiple copies of M on Y (Hediger *et al*., 1998). 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 houseflies (Bull & Charnov, 1977). 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 & Wada, 1989) or occasionally on X (Schmidt *et al*., 1997). In these A^M (or X^M) strains, females are XX and males are also XX (or XO) (Hiroyoshi, 1964; Wagoner, 1969; Franco *et al*., 1982; Denholm *et al*., 1983, 1990). The M located on Y is thought to be the same factor as the M located on any of the other autosomes (Tomita & Wada, 1989; Schmidt *et al*., 1997). Clines in the relative frequency of Y^M and A^M males have been reported from the USA (Hamm *et al*., 2005), Japan (Tomita & Wada, 1989) and Europe (Franco *et al*., 1982; Kozielska *et al*., 2008), with Y^M males being more common with increasing latitude (and in some cases altitude), and the cline in Europe appears to be stable (Kozielska *et al*., 2008).

Populations that contain males with multiple M factors (III^M/III;XY^M for example) or males homozygous for an A^M factor (e.g. III^M/III^M) also contain an F^D factor (also known as F) to produce females. F^D is epistatic to M (Dübendorfer & Hediger, 1998) and has never been detected in houseflies from North America. F^D is located on the fourth chromosome (McDonald *et al*., 1978; Cakir, 1999) and produces

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females even in the presence of up to three M factors (McDonald *et al*., 1978; Schmidt *et al*., 1997; Hediger *et al*., 1998). F^D has recently been sequenced (D. Bopp, personal communication). Unfortunately, the sequence of M has not yet been determined.

Populations with autosomal males may produce a variety of sex ratios depending on the number of M factors present in the males and the frequency of F^D in females. According to Fisher's theory, the optimal sex ratio is 1 : 1 due to the concept of random mating, because if one sex is rare it will have greater reproductive success (Fisher, 1958; Goodenough *et al*., 1993). Therefore, the only stable situation is for parents to produce equal numbers of male and female offspring, and any deviation should be automatically corrected (to 1 : 1) with selection (Fisher, 1958). Housefly populations that contain males with a single M factor will produce offspring with a 1 : 1 sex ratio. This 1 : 1 ratio can be found if males are XY^M or A^M/A. If a male is A^M/A^M, only male offspring are produced (in the absence of F^D). In some populations, males may carry multiple M factors, which would again produce an excess of male offspring in the absence of F^D. A male heterozygous for M on two linkage groups will produce offspring with a 3 : 1 ratio of males to females. The 7 : 1 male/female ratio is produced when three M factors exist in heterozygous form. These situations all assume that the female does not carry the F^D factor.

Despite the relatively high mobility of houseflies (Schoof & Siverly, 1954), and the presence of males with either XY^M or III^M/III in populations in New York and North Carolina (Hamm *et al*., 2005), not all populations have both XY^M and III^M/III males. For example, all male flies in Maine (2002) were XY^M (Hamm *et al*., 2005). Conversely, male flies collected from Florida in 1973 (McDonald *et al*., 1975) and 2002 (Hamm *et al*., 2005) were all III^M/III. However, flies in neighbouring Alabama (Marshall County) collected in 1998 were XY^M (Liu & Yue, 2001). Migration between Alabama and Florida seems likely, but no Y^M males have been found in Florida over a 30-year time period, suggesting a selective advantage for III^M/III males in Florida. However, only one study has examined the changes in frequency of A^M versus Y^M males over time in field populations (Kozielska *et al*., 2008) and no studies have examined laboratory strains.

Herein, the frequency of A^M and Y^M males in the North Carolina population was re-evaluated after being in the laboratory and the field. The present study reveals that the frequency of Y^M and III^M (and even males with two M factors) can change very rapidly in the laboratory, but that changes are much slower in field populations. The frequency of F^D in the laboratory and field populations was also determined.

2. Materials and methods

(i) *Housefly strains*

The NC 2002 strain was collected in 2002 from a dairy in Wake County, North Carolina (Hamm *et al*., 2005), and has been reared under standard laboratory conditions (see below). The NC 2006 and NC 2007 strains were established with greater than 400 pupae collected (from the same location as NC 2002) in July 2006 and May 2007, respectively. The NC Lab 02:06 strain was created with flies from the NC 2002 collection that have remained in the laboratory from 2002 until 2006. A minimum of 800 flies were used to start each new colony cage (i.e. each generation). The aabys strain, 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 to determine the linkage of M.

All flies and larvae were kept at 28 °C with a 12 : 12 h light/dark photoperiod. Housefly larvae were reared on a medium prepared with 1·8 litres of water, 500 g calf manna (Manna Pro Corp., St. Louis, MO), 120 g bird and reptile litter wood chips (Northeastern Products Corp., Warrensburg, NY), 60 g dried active baker's yeast (MP Biomedicals, Solon, OH) and 1210 g wheat bran (Cargill Animal Nutrition, Minneapolis, MN). Adult fly colonies were kept in mesh cages ($35{\cdot}6 \times 25{\cdot}4 \times 26{\cdot}7$ cm^3) provided with a 1 : 1 mix of sugar and powdered milk and water *ad libitum*.

(ii) *Linkage of M*

To determine the linkage of M, a backcross experiment was carried out as previously described (Hamm *et al*., 2005). One to four day old male flies (from NC Lab 02:06, NC 2006 or NC 2007 strains) were individually crossed with 3–6 unmated aabys females (2–5 days old). Flies were kept in 270-ml paper hot cups (International Paper; Post Turbhe, Navi Mumbai, India) with polychiffon tops and were fed with granulated sugar/powdered milk (1 : 1) for 3 days. Water was provided using saturated cotton. After 3 days, flies were placed into cups with media (see above) to oviposit, and were provided cotton soaked in a 10% sugar water solution. Media cups were changed every other day for 7 days. Media cups with eggs were stirred and additional medium was provided on the day adult flies were removed. Cups were misted with distilled water daily for 4 days.

Emerging F_1 males and females were counted. Three F_1 males from each original male were individually used in a backcross with 3–6 aabys females as described above. If the F_1 ratio was 1 : 0 (males/females), then eight backcrosses were made. The emerging backcross individuals were phenotyped according to sex and markers. XY^M males were

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Table 1. *Female housefly genotypes of the NC Lab 02:06 and NC 2007 strains, identified by F_1 ratios produced. Both strains were crossed with NC Lab 02:06 males*

Genotype			% of population	
Male[a] (NC Lab 02:06)	Female[b]	Male/female ratio	NC Lab 02 : 06	NC 2007
X^M/X	$F^D/F;X^MX$	1 : 1·67	24·5%	0%
X^M/X	$F^D/F;XX$	1 : 3	9·4%	0%
X^M/X	$F^D/F;X^MX^M$ or $F/F;XX$	1 : 1	54·7%[c]	62·5%[c]
X^M/X^M	F^D/F (unknown X)	1 : 1	7·6%	4·2%
X^M/X^M	$F/F;XX$	1 : 0	3·8%	33·3%

[a] Assumed to be X^M/X (or X^M/X^M) due to the presence of X^M in females, but could also be XY^M or X^MY^M.
[b] *F* is located on autosome IV.
[c] Most females were likely *F*/*F*;XX (see the text for an explanation).

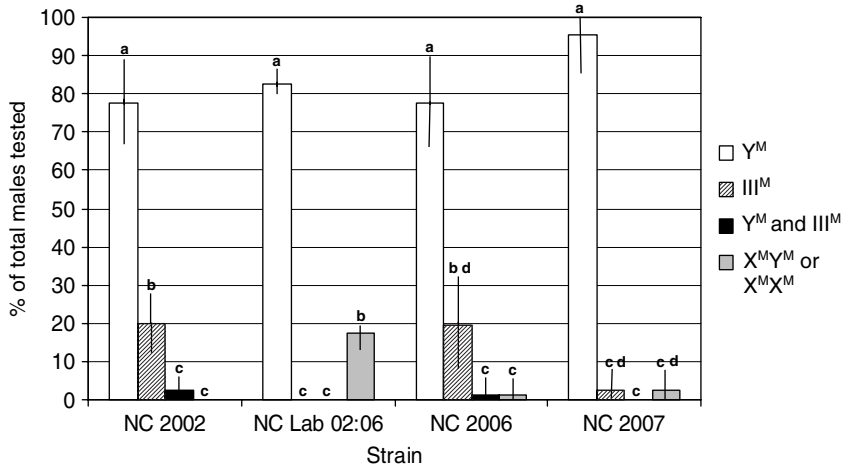

Fig. 1. Linkage of the housefly male determining factor, M, over time under two environmental conditions. The results for NC 2002 have been reported previously (Hamm *et al.*, 2005) and are given here for reference. Error bars represent the standard deviations. Results with different letters are significantly different ($P = 0{\cdot}05$).

identified by the lack of association between sex and the autosomal markers, whereas III^M/III males were identified by backcross females being brown body and males being wild-type.

A *t*-test was performed for pairwise comparisons of the means for each linkage group from all strains tested. A significant *P*-value ($\leqslant 0{\cdot}05$) indicated that the means were significantly different.

(iii) *Frequency of* F^D

To determine the frequency of F^D, one NC Lab 02:06 male was crossed with one aabys female and one NC Lab 02:06 (or NC 2007) female. Both females were left with the male for at least 4 days. After day 4 the males were removed and each female was individually placed into a cup with media (see above) 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 at least 7 days. Media cups with eggs were stirred and additional medium was provided the day adult flies were removed. Cups were misted with distilled water daily for 4 days.

The F_1 males and females were counted for each female. The aabys♀ × NC Lab 02:06♂ crosses that produced all male offspring identified the male as A^M/A^M. If the NC female that was crossed with the same male produced male and female offspring, then she carried an F^D factor. F_1 sex ratios from each of the above crosses used for F^D determination were used to calculate a χ^2 value relative to the expected possible ratios (1 : 1, 1 : 1·67 and 1 : 3) (Table 1). If the χ^2 value showed significance for one ratio, the data were used to determine female genotype. In rare cases where no ratio was significant or two or more ratios showed significance, the data were excluded.

3. Results

(i) *Linkage of M*

The NC Lab 02:06 F_1 progeny (aabys♀ × NC Lab 02:06♂) in 12 out of 69 crosses (with $\geqslant 10$ offspring)

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produced no daughters, indicating these 12 males are homozygous for M. There was no association between sex and marker found in any of the backcross progeny (aabys♀ × F_1 (aabys♀ × NC Lab 02:06♂)) (60/79 with $n \geqslant 100$ phenotyped). The F_1 result combined with the backcross data indicates that 17·4% of the males in this strain are X^MY^M or X^MX^M with 82·6% being XY^M or X^MX (Fig. 1). We were unable to distinguish Y^M from X^M in males with our crosses, as no marker is known for the X or Y chromosomes in houseflies. The differences between the NC 2002 and NC Lab 02:06 strains are unlikely to be due to genetic drift as a minimum of 800 flies were used to start each new colony cage (i.e. each generation).

The NC 2006 F_1 progeny (aabys♀ × NC 2006♂; 102 out of 110 crosses produced offspring) had only one individual that produced all male offspring (35 males, 0 females). Eight of 102 crosses produced a male/female sex ratio of $>0{\cdot}70$ and $<1{\cdot}0$. When male percentages were 75% or greater, 6–9 of the male offspring were individually used for the backcross generation. The backcross (aabys♀ × F_1 (aabys♀ × NC 2006♂)) offspring were phenotyped, and revealed 77·8% XY^M, 19·4% III^M/III, 1·4% $III^M/III;XY^M$ and 1·4% X^MY^M (or X^MX^M) (Fig. 1). The X^MY^M (or X^MX^M) determination was based on the F_1 result of all male offspring and the backcross not associating with a marker. III^M/III males are determined by males having black bodies (+/*bwb*) and females being brown (*bwb*/*bwb*). The $III^M/III;XY^M$ male was determined by a skewed sex ratio in the F_1 (0·84) and the backcross showing only brown-bodied females, but males that were either brown-bodied or wild-type. Emergence was 82% with $\geqslant 50$ phenotyped individuals used in backcross determinations for this strain.

Only one male in the NC 2007 strain produced all males in the aabys♀ × NC 2007♂ F_1. Analyses of the backcross progeny revealed that M was most commonly (95·3%) linked to Y (i.e. not associated with an autosomal maker), with 2·3% males being III^M/III and 2·3% being X^MY^M (or X^MX^M).

(ii) *Frequency of* F

Females from the NC Lab 02:06 population contained F^D factors at a frequency of 41·5% ($n \geqslant 50$) (Table 1). This is likely an underestimate of the actual frequency due to the 1 : 1 ratio produced by $F/F;XX$ or $F^D/F;X^MX^M$ females when mated with a X^MX male, which made up 54·7% of the NC Lab 02:06 population. A further underestimation is caused by crosses that produced a significant χ^2 for both 1 : 1·67 and 1 : 3 ratios. Both ratios indicate F^D females, but the genotypes cannot be determined and, therefore, are not included in the data. The relatively high frequency of F^D was as expected due to the frequency of homozygous males found in the population.

The NC 2007 field-collected females produced only 1 : 1 or 1 : 0 F_1 (NC 2007♀ × NC 02:06♂) male/female ratios. Emergence was 68·8% from the 109 crosses started. Females that were crossed with X^MX^M males (NC 02:06) were primarily $F/F;XX$ with a low frequency of F^D/F detected (Table 1). Crosses with males (NC 02:06) having only one copy of M (Table 1) revealed that 62·5% of the females were $F/F;XX$ or $F^D/F;X^MX^M$. These females are most likely to be $F/F;XX$ because of the low frequency of F^D/F found in the other females from this population and that only one X^MX^M male was found. Given the low number of males with more than one M factor (Fig. 1), the relatively low frequency of F^D females in NC 2007 (Table 1) was as expected.

4. Discussion

Comparison of the starting population (NC 2002) with the colony after being reared in the laboratory for 4 years shows that the population can change rapidly, as the III^M and $III^M + Y^M$ males became undetectable, and the X^MY^M (or X^MX^M) males became 18% of the population. In contrast, houseflies field-collected in 2006 remained approximately the same as they had been in 2002, with the exception of one X^MY^M (or X^MX^M) that was detected in 2006, but not 2002.

The NC 2007 collection showed a decrease in the number of III^M males. It is unclear what could be responsible for the decline in III^M males. Temperature does not appear to be involved as the average high and low temperatures in 2006–2007 were very similar (results not shown).

The appearance of X^MY^M (or X^MX^M) males after 4 years of laboratory rearing was unexpected as these males were undetected in 2002. This suggests that either these males were rare in 2002 (below the detection level) or that there was a transposition of M (presumably from Y) to X, leading to the production of an X^MY^M male. The detection of X^MY^M males in the NC 2006 and NC 2007 collections suggests that X^MY^M males were likely present in NC 2002 at a low frequency. This would imply that F^D was also found in females of the NC 2002 collection and is supported by F^D females being found in the NC Lab 02:06 strain. The fact that F^D females probably existed in the NC 2002 collection indicates that two systems are interacting (XX versus F^D females and autosomal versus Y males) to cause the differences seen between NC 2002 and NC Lab 02:06 strains, and that X^MX^M males have an advantage (relative to $Y^M + III^M$ males) and/or $F^D/F;X^M/X$ females have an advantage (relative to XX females). Not knowing the original frequency of F^D in the NC 2002

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population makes it difficult to choose between these scenarios.

These results highlight three important conclusions, especially for comparison of the NC 2002 and NC 2006 collections. First, although under some conditions (i.e. laboratory rearing) the polymorphisms in male determination can change over time, in field populations they are relatively stable. This agrees with the recent report on houseflies from Europe where a cline in Y^M versus A^M flies was found to be relatively unchanged after 25 years (Kozielska *et al*., 2008). Secondly, some of the male determining genotypes are rare and will remain undetected unless a sufficient number of males are evaluated. Thirdly, it is important to determine male and female genotypes on houseflies that have not been kept in the laboratory for many generations, as the results will be influenced by changes that can occur during laboratory rearing.

Little is known about the selective advantages or disadvantages between Y^M and A^M males. We know from previous studies that III^M/III males have a selective advantage in Florida and have been stable there for the past 30 years (McDonald *et al*., 1975; Hamm *et al*., 2005). It appears that X^MY^M (or X^MX^M) males are selected against in some field populations, but are at an advantage under laboratory rearing conditions. For example, these males were undetectable in NC 2002 and were at a very low frequency in 2006 and 2007. However, 17·4% of the males were X^MY^M (or X^MX^M) after 4 years of laboratory rearing. Fitness studies will need to be carried out to help determine what factors allow III^M/III to have an advantage in one location (Florida) and XY^M to have an advantage in another location (Maine) or under other conditions.

The presence of F^D has always been found within populations containing males with homozygous M factors and/or multiple M factors (McDonald *et al*., 1978; Denholm *et al*., 1985, 1990; Tomita & Wada, 1989; Cakir, 1999; Kozielska *et al*., 2008), although F^D had not been reported previously in houseflies from the USA. In Japan, the frequency of F^D ranges from 0 to 0·99 (Tomita & Wada, 1989). The NC Lab 02:06 population had 17·4% homozygous males with a minimum of 41·5% of the females carrying F^D. It is possible that this population might fix for homozygous males and that females would become the heterogametic sex in this population owing to the high frequency of females with F^D that also carry M, which will increase the production of homozygous males. This is in contrast with the field population where a few homozygous males are found (1·4–2·3%) and fewer F^D females occur (4·2%). It would be of interest to determine the frequency of F^D in housefly populations that have no detectable frequency of A^M/A^M or (A^M/A) males. This now appears feasible to do, as a PCR assay has been developed that can differentiate between F/F and F^D/F females (Kozielska *et al*., 2008).

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