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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(Received 1 August 2008 and in revised form 19 October 2008)

**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 $X^M/Y^M$. 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 $X^M/Y^M$ 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...
females even in the presence of up to three M factors (McDonald et al., 1978; Schmidt et al., 1997; Hediger et al., 1998). FD 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 FD 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 XYM or AM/A. If a male is AM/A, only male offspring are produced (in the absence of FD). In some populations, males may carry multiple M factors, which would again produce an excess of male offspring in the absence of FD. 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 FD factor.

Despite the relatively high mobility of houseflies (Schoof & Sively, 1954), and the presence of males with either XYM or III/M/III in populations in New York and North Carolina (Hamm et al., 2005), not all populations have both XYM and III/M/III males. For example, all male flies in Maine (2002) were XYM (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 XYM (Liu & Yue, 2001). Migration between Alabama and Florida seems likely, but no Y 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 AM versus Y males over time in field populations (Kozielska et al., 2008) and no studies have examined laboratory strains.

Herein, the frequency of AM and Y 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 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 FD 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, aristopedia, 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 (35·6 × 25·4 × 26·7 cm³) 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 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 F₁ males and females were counted. Three F₁ males from each original male were individually used in a backcross with 3–6 aabys females as described above. If the F₁ ratio was 1:0 (males/females), then eight backcrosses were made. The emerging backcross individuals were phenotyped according to sex and markers. XYM males were
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 (\( \leq 0.05 \)) 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 \( \varphi \times \) NC Lab 02:06 \( \varphi \) crosses that produced all male offspring identified the male as AM/AM. 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 \( \chi^2 \) value relative to the expected possible ratios \( (1:1, 1:1.67 \text{ and } 1:3) \) (Table 1). If the \( \chi^2 \) 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 \( \varphi \times \) NC Lab 02:06 \( \varphi \)) in 12 out of 69 crosses (with \( \geq 10 \) offspring) were removed. Cups were misted with distilled water daily for 4 days.

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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♂ × F₁ (aabys♀ × NC Lab 02:06♂)) (60/79 with n ≥ 100 phenotyped). The F₁ result combined with the backcross data indicates that 17.4% of the males in this strain are XMYM or XMXM with 82.6% being XYM or XMX (Fig. 1). We were unable to distinguish YM from XM 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 F₁ 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 0.70 and < 1.0. When male percentages were 75% or greater, 6–9 of the male offspring were individually used for the backcross generation. The backcross (aabys♀ × F₁ (aabys♀ × NC 2006♂)) offspring were phenotyped, and revealed 77.8% XYM, 19.4% III/M, 1.4% III/M;XYM and 1.4% XMXM (or XMX) (Fig. 1). The XMYM (or XMXM) determination was based on the F₁ result of all male offspring and the backcross not associating with a marker. III/M males are determined by males having black bodies (+/bwb) and females being brown (bwb/bwb). The III/M;XYM male was determined by a skewed sex ratio in the F₁ (0:84) and the backcross showing only brown-bodied females, but males that were either brown-bodied or wild-type. Emergence was 82% with ≥ 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♂ F₁. 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 XMYM (or XMXM).

(ii) Frequency of F

Females from the NC Lab 02:06 population contained FD factors at a frequency of 41.5% (n ≥ 50) (Table 1). This is likely an underestimate of the actual frequency due to the 1:1 ratio produced by F/F;XX or FD/F;XM♂ females when mated with a XM♂ male, which made up 54.7% of the NC Lab 02:06 population. A further underestimation is caused by crosses that produced a significant χ² for both 1:1:67 and 1:3 ratios. Both ratios indicate FD females, but the genotypes cannot be determined and, therefore, are not included in the data. The relatively high frequency of FD 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 F₁ (NC 2007♀ × NC 02:06♂) female/female ratios. Emergence was 68.8% from the 109 crosses started. Females that were crossed with XMYM males (NC 02:06) were primarily F/F;XX with a low frequency of FD/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 FD/F;XM♂. These females are most likely to be F/F;XX because of the low frequency of FD/F found in the other females from this population and that only one XM♂ male was found. Given the low number of males with more than one M factor (Fig. 1), the relatively low frequency of FD 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 males became undetectable, and the XMYM (or XMXM) 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 XMXM (or XMXM) 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 XMYM (or XMXM) 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 XMYM male. The detection of XMYM males in the NC 2006 and NC 2007 collections suggests that XMYM males were likely present in NC 2002 at a low frequency. This would imply that FD was also found in females of the NC 2002 collection and is supported by FD females being found in the NC Lab 02:06 strain. The fact that FD females probably existed in the NC 2002 collection indicates that two systems are interacting (XX versus FD females and autosomal versus Y males) to cause the differences seen between NC 2002 and NC Lab 02:06 strains, and that XMYM males have an advantage (relative to YM+III/M males) and/or FD/F;XM♂ females have an advantage (relative to XX females). Not knowing the original frequency of FD in the NC 2002
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 acline 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 \( X^M Y^M \) (or \( X^N X^M \)) 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 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.

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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).

We thank Dr Wes Watson for collecting the houseflies, the State Climate Office of North Carolina for weather data, Cheryl Leichter, Juliane Deacutis and Tomás Lazo for technical assistance and Drs. Don Rutz and Andy Clark for valuable discussions.

References


