Measuring the duration and frequency of wing beat of *Musca autumnalis* (Diptera: Muscidae) using a novel tether method

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Abstract—The wing beat frequencies (WBF) and flight durations of the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae), were evaluated at 1, 3, 5, 7, 11, and 14 days post-eclosion. For flight tests, flies were tethered magnetically using magnetic primer paint. WBF were measured stroboscopically. The average WBF for one-day-old flies was significantly lower compared with the average WBF of all other age groups for both female and male face flies. Based on our results, male and female face flies require more than 24 hours post eclosion to reach a WBF of over 167 beats per second and continuously fly for more than 10 minutes. Age was a significant factor towards WBF. The present study is the first to report laboratory descriptions of face fly flight capabilities. The benefits of the magnetic paint tether (MagPaT) method are discussed.

Résumé—Nous avons évalué les fréquences de battement des ailes (WBF) et la durée du vol chez la mouche faciale, *Musca autumnalis* De Geer (Diptera: Muscidae), 1, 3, 5, 7, 11 et 14 jours après l'éclosion. Durant les essais en vol, les mouches étaient retenues magnétiquement à l'aide d'une peinture d'apprêt magnétique. Les fréquences de battement des ailes ont été mesurées par stroboscopie. La WBF moyenne des mouches d'un jour est significativement inférieure à celles des moyennes des autres groupes d'âge de mouches faciales femelles et mâles. D'après nos résultats, il faut aux mouches faciales mâles et femelles plus de 24 heures après l'éclosion pour atteindre une WBF supérieure à 167 battements par seconde et pour voler continuellement plus de 10 minutes. L'âge est un facteur significatif en ce qui a trait à la WBF. Notre étude est la première à décrire en laboratoire les capacités de vol des mouches faciales. Nous discutons des avantages à utiliser la méthode de rétention avec une peinture d'apprêt magnétique.

The face fly, *Musca autumnalis* De Geer (Diptera: Muscidae), is a pest of beef cattle, and larvae develop in fresh cattle dung. In areas where face flies are prevalent, they can be found on the faces of pastured cattle and on objects surrounding the pastures such as vegetation and fences (Kaya and Moon 1978). Face flies have a diurnal activity pattern, where they disperse away from cattle to trees surrounding the pastures in the evening and return to the pastures in the daylight hours (Killough *et al.* 1965; Pickens and Nafus 1982). Mark-release-recapture studies have shown that face flies are capable of dispersing distances of 3 km over a 24-hour period (Killough *et al.* 1965). These studies indicate that face flies are strong fliers. However, there are no reports of descriptive information on the flight capabilities, such as duration of uninterrupted flight and wing beat frequency (WBF) for face flies. Such information may reveal whether the reported dispersal distances and behaviour for face flies are limited by the ability of the face flies to continuously fly over long periods of time.

It is possible, however, for such flight capabilities to deteriorate as face flies age. Krafsur *et al.* (1995) have determined the mean life expectancy, for naturally occurring face fly populations, to be 11 days for females and 10 days for males. Differences in flight capabilities

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have been documented among aging house flies (*Musca domestica* Linnaeus) (Diptera: Muscidae) (Rockstein and Bhatnagar 1966). We hypothesised that face flies would exhibit similar changes in flight capabilities as they near their life expectancy. The objective of this study is to characterise the duration and WBF among face flies of different age classes. We introduce the magnetic paint tether (MagPaT) method as a novel way to tether and evaluate insects in flight.

This investigation was conducted between June and August 2009. All flies examined in this experiment were collected as larvae in cattle dung pats from the Sierra Foothills Research and Extension Center in Browns Valley, California, United States of America.

Puparia from field-collected dung pats were stored individually in portion cups and kept in an incubator (25 °C, 23 ± 2% relative humidity, 16 hours light: 8 hours dark). Cups were checked daily for eclosion. Within 24 hours of eclosion, 10 male and 10 female flies from the same emergence date were released into a cage. Six cages were set up and each cage of flies was assigned an age at which that cohort of flies was to be tested. Flies were tested at 1, 3, 5, 7, 11, and 14 days after eclosion. The cages were held between 23 °C and 25 °C (29 ± 2% relative humidity, 16 hours light: 8 hours dark). Flies in each cage were provided water, sugar cubes, powdered skim milk, fresh beef liver, and cattle dung (for oviposition) *ad libitum*.

To prepare flies for flight tests, the cage of flies to be tested was placed in a refrigerator (4 °C) for about three minutes to immobilise them. This step eliminated bias towards measuring only flies that could be easily caught. Five flies of each sex were arbitrarily chosen to be tested. Instead of using glue or other adhesives to tether the insects, a small dot (~0.5 mm diameter) of Rust-Oleum® Specialty Magnetic (Rust-Oleum Corporation, Vernon Hills, Illinois, United States of America) latex primer paint was applied to the mesonotum of each anesthetised fly. This paint makes surfaces attractive to magnets. Flies were allowed to recover individually beneath a transparent film canister. Within five minutes, the paint dried and the flies resumed activity. To remove a fly from the canister, a 50 mm diameter magnet was placed underneath the opening of the canister and flipped over. In most cases, the fly would attach to the magnet by their mesonotum and was manually transferred to a 1.6 mm × 1.6 mm (height × diameter) cylindrical, neodymium magnet that was placed at the tip of a 2 cm iron nail. This fly-magnet-nail unit was placed onto a second, larger neodymium magnet, which was attached to a steel beam. In our case, each unit was attached to a 17 mm × 9 mm (base × height) triangular magnet. All flight tests were conducted between the hours of 0900 and 1400 at 24 °C and 26 °C (27% ± 2% to 40% ± 2% relative humidity).

Flies that did not begin flying immediately were stimulated to do so by placing a finger underneath their legs and removing the finger after first fly leg contact. Flies that failed to continuously fly for at least one minute after three stimulation attempts were removed and noted. The initial wing beat frequency within the first 30 seconds of suspension was measured stroboscopically (held ~7 cm away). Once the initial measurements were taken, the flies were flown to exhaustion. Tethered flies were checked every five minutes, a method used by Rockstein and Bhatnagar (1966). Flies that stopped flying during observations were stimulated with the same technique used for flies that failed to fly initially. Flies that failed to fly continuously for at least one minute were considered to be exhausted. Up to five flies were observed at once.

Face flies collected from the field can harbour infections by the nematode *Paraiotonchium autumnale* (Nickle) (Tylenchida: Iotonchiidae), and therefore all tested flies were dissected afterwards and examined for the presence of nematodes. Any parasitised flies found were removed from the analysis.

Of the 30 female flies examined, one one-day-old fly and two five-day-old flies failed to fly, and one three-day-old fly and one seven-day-old fly were parasitised by *P. autumnale*. For the 30 male flies examined, one 11-day-old fly was parasitised, three flies failed to fly. One 14-day-old male fly that flew for 430 minutes, was considered an outlier, and was excluded from the statistical analysis.

Results were analysed using two-way ANOVA ($\alpha = 0.05$) to evaluate the effects of age and sex of the fly on WBF and on flight duration in JMP® 9.0 (SAS 2010). The analysis

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Fig. 1. Mean female (F) and male (M) face flies initial WBF versus age. Initial WBF for one-day-old flies was significantly lower than the rest of the age groups.

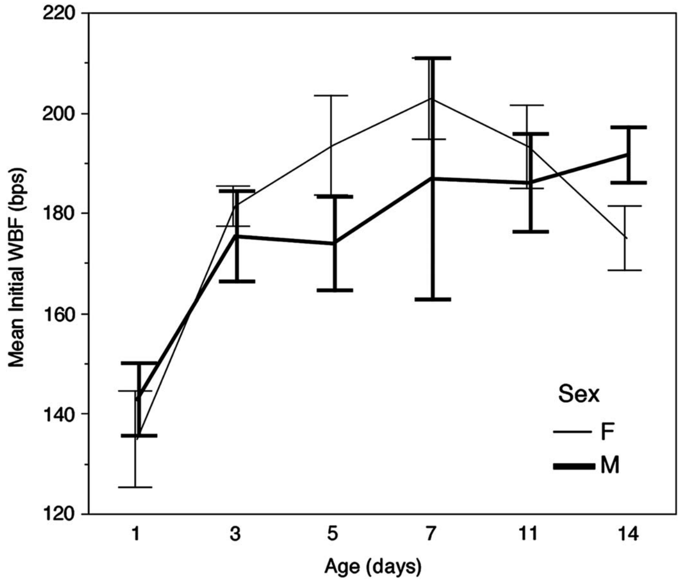
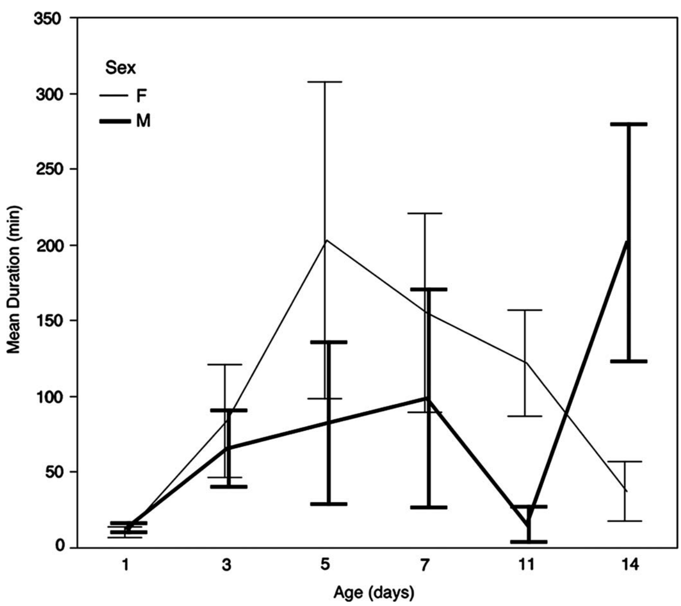


Fig. 2. Mean female (F) and male (M) face flies flight duration versus age. No significant differences were found across age and sex.



revealed a significant main effect for the age of flies, $F(5,41) = 9.856$; $P < 0.001$; $R^2 = 0.58$; MSE = 353.6. Tukey's honestly significant difference (HSD) test showed that one-day-old flies had a significantly ($\alpha = 0.05$) lower WBF than flies of all other age groups. The main effect for sex of flies was not significant, $F(1,41) = 0.40$; $P = 0.5329$. The interaction between age and sex

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of flies was also not significant, $F(5,41) = 1.17$; $P = 0.3384$. For flight duration, age and sex were not significant factors ($F(5,41) = 1.947$; $P = 0.1073$ and $F(1,41) = 0.002$; $P = 0.9647$, respectively). The *P*-value for the interaction effect between age and sex on flight duration was 0.0526 ($F(5,41) = 2.41$). The direction of the interaction effect leans towards a significant relationship, but improvements to the current experimental design are needed to truly determine the direction of the relationship.

We found that *P. autumnale*-parasitised flies are capable of tethered flight, but there were not enough individual flies to assess whether or not parasitism has debilitating effects on face fly flight capabilities. Chirico (1996) reported reduced flight capacities for *P. autumnale*-exposed face flies, but details of the study were not stated. This is a topic that needs further investigation.

Our study found that female and male face flies are capable of sustaining tethered flight for over an hour after 72 hours post eclosion. The results support the finding of Killough *et al.* (1965) that face flies are strong fliers. Our study also found that one-day-old female and male face flies need additional time to reach their maximum WBF (Fig. 1). This result is similar to what Rockstein and Bhatnagar (1966) found when investigating the flight capabilities of tethered aging house flies. They also reported a trend where flight duration for male and female house flies increased initially and dropped as flies became senescent. They attributed the differences to the physiological changes in the flight muscles as house flies age. There were no significant differences in flight duration for male and female face flies across the age groups (Fig. 2). Physiological differences between age groups may have been present, but uninterrupted flight may not be a natural behaviour for face flies of all ages. While face flies are capable of dispersing over long distances, perhaps this is achieved by resting on objects in the field along the way.

While this study is not the first to use some kind of magnetic system to tether flies for flight studies, to our knowledge, this is the first study to use magnetic paint. Duistermars and Frye (2008) developed a method of having *Drosophila* Fallén (Diptera: Drosophilidae) affixed to magnets by attaching metal minuten pins using ultraviolet-activated glue on the thorax. Our method eliminates the need for glue or any other additional adhesives to make insects attractive to magnets. We have found several advantages of using magnetic paint for investigations involving tethered insect flight. First, while water-based, non-toxic glues can be used to tether small insects such as *Drosophila* flies and aphids (Hemiptera: Aphididae), stronger, fast-drying glues (*e.g.*, using volatile solvents) are usually required to tether larger, stronger-flying insects such as house flies and face flies. Strong glues can potentially harm the insect and/or cause accidental adhesion of appendages of the insect. The magnetic latex paint used in our study is non-toxic, and there was low risk of accidental adhesion to other parts of the body of the insect. Second, while it is possible to conduct repeated flight studies with the same insects using glues, the constant removal and re-application of the material can damage the insects and may render them unsuitable for future testing. The MagPaT method eliminates the need for constant reapplication and removal of adhesives. In our study, once a face fly was flown to exhaustion, they were simply removed from the magnet (with the drop of paint intact on the mesothorax) with little effort. Depending on the application, the use of the MagPaT method may reveal additional advantages over using glues and other adhesives to tether insects in flight studies.

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