

Female Choosiness and Tracking Approaches in  
*Drosophila*

COMPLEX MRES CASE PRESENTATION 1

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### **Abstract**

During courtship in *Drosophila* species-specific auditory information is signalled to the female via the males courtship song. This sexually selected behaviour is interesting from an evolutionary and an anatomical level. This report will consider quantifying female choosiness in *Drosophila* with the goal to understand courting behaviour more comprehensively. Two methods for tracking flies using Wolfram *Mathematica* 9 are developed and their application outlined.

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# 1 Introduction

*Drosophila*, like many other species, exhibit sexually selected behaviour when courting. Studies into their courtship behaviour are well established, beginning with work by Strutevant (1915) and Bastock & Manning (1955), and progressing to machine-vision programs to track and classify types of behaviour computationally (such as Ctrax<sup>1</sup>, see Branson *et al*, 2009).

Courtship in *Drosophila* can last for several minutes, which allows time for the female to decide how fit the male is and also to evaluate his species (Ferveur, 2010). The females' degree of "choosiness" cannot be seen as the sole cause of variation in courtship time as quick matings could actually reflect forced copulation (Manoli & Baker, 2004). Hence, to quantify female choosiness it is important to consider all the interactions of a courting pair, which is where tracking programs become invaluable.

Male courtship songs are species-specific and regarded as a central part of premating barriers (Riabinina *et al*, 2011), therefore the songs and the ears of *Drosophila* have been extensively studied (summarised by Tauber & Eberl (2003)). This project aims to link some of the gaps between experiments analysing the songs of *Drosophila* and experiments analysing their overall courtship behaviour. A tracking program that could be used with videos taken for the purpose of audio recording is suggested.

The first section will review mechanisms for the evolution of sexual selection, as well as the three key modelling frameworks used (population genetics, quantitative genetics and invasion analysis). The second section will describe some details of courtship in *Drosophila*, and look at how the females ear is adapted to hear the songs of her conspecific best. The third section will discuss some experiments tried to quantify the amount of choosiness a female will exhibit during courtship. Finally, the fourth section will discuss the use and implementation of tracking courting flies from videos.

## 2 Sexual Selection

Sexual selection arises from variance in mating success, as opposed to variance in individual survival (Lande, 1981). Sexually selected traits can maximise reproductive success by increasing the chances of winning intermale competitions (intrasexual selection) or by making the male more attractive to the female (intersexual selection). Intersexually selected traits can often be disadvantageous for the male, for example by making them more visible to predators (such as the extravagant plumage in peacocks) or by having a large energy cost to sustain (such as exaggerated horns), and could lead to a species' extinction. Hence it is difficult to explain the evolution of disadvantageous characteristics for one sex and the corresponding preference in the other sex. This problem becomes even less clear when the female receives little more from the mate than his sperm (Kirkpatrick & Ryan, 1991). A solution to this problem can be assisted by considering the costs and benefits for a female to have a preference for a particular trait.

### 2.1 Costs and Benefits to Choosiness

There are several costs and benefits associated with the evolution of female choosiness. Choosiness can be costly if the female is exposed to predators for longer amounts of time

<sup>1</sup><http://ctrax.sourceforge.net/>

whilst searching for mates or if mating is harmful to the female. Furthermore females run the risk of ending up unfertilized by being choosy (Kokko & Mappes, 2005). Benefits of choosiness can be direct and indirect for the female.

### 2.1.1 Direct Benefits

There will be a direct benefit when choosiness is related to the females survival and fecundity. An example of this is the good-parent process (Price *et al*, 1993 and Iwasa & Pomiankowski, 1999), where females prefer males that advertise their parental ability. Also in situations where there are mating-related costs, such as seminal toxins (Rice, 1996) or damage by male genital spines (Arnqvist & Rowe, 2005), choosiness (and therefore less harmful mating) can be directly beneficial to the female.

### 2.1.2 Indirect Benefits

The indirect benefits of female choosiness are to produce offspring with a higher reproductive value (Kokko *et al*, 2002). Choosiness in females could evolve because of indirect benefits via two mechanisms; Fisher's runaway process or the good-genes model.

Via the Fisher Process there is the benefit of having attractive sons and therefore more grandoffspring. Fisher (1915) proposed that female preference for a male trait can evolve because of linkage disequilibrium with the male trait. If the preference is present in the female, then they will tend to mate with males with the trait, therefore their offspring will tend to have both the preference and trait alleles. Hence positive selection for the trait will induce selection on the preference due to their association, and this in turn will create stronger selection for the trait, a self-reinforcing runaway process.

Via a "good-genes" hypothesis females may have a preference for males which advertise heritable quality i.e. enhanced offspring reproductive value (Kokko, 2001 and Kokko *et al*, 2002). Hamilton & Zuk (1982) suggested a good-genes hypothesis in terms of parasites, where there is a correlation between parasite resistance genes and male trait expression which leads to a correlation between the female preference and resistance genes.

Additionally females may prefer different types of males depending on their own genotype to produce offspring from compatible genes. There can also be no benefits to choosiness; preference can evolve as a by-product of natural selection (Kirkpatrick 1987, Kirkpatrick & Ryan 1991). Figure 1 shows a summary of the mechanisms for the evolution of mating preferences taken from Kirkpatrick & Ryan (1991).

## 2.2 Models of Sexual Selection

The four main modelling frameworks to describe sexual selection are population genetics, quantitative genetics, invasion analysis and individual-based simulations (Kuijper, Pen & Weissing, 2012). I will give a brief review of the first three of these models and illustrate how they can describe the evolution of female preference by application to the Fisher runaway process. Comparisons between these frameworks and a more in-depth analysis is discussed by Kuijper, Pen & Weissing (2012).

1. Direct selection of preferences
  - A. Males provide resources to females or offspring
  - B. Costs of searching for mates
  - C. Selection against hybridization
  - D. Males differ in sperm fertility
  - E. Pleiotropic effects of preference genes
  - F. Disease or parasite transmission
2. Indirect selection of preferences
  - A. Runaway process
  - B. Good genes
    - (i) Host-parasite coevolutionary cycles
    - (ii) Unconditionally advantageous mutations
    - (iii) Unconditionally deleterious mutations
  - C. Genetic epistasis and dominance
  - D. Social system
  - E. Mutation pressure on trait
3. Other mechanisms
  - A. Random genetic drift
  - B. Group selection
  - C. Mutation pressure on preference

Figure 1: Proposed mechanisms for the evolution of mating preferences in polygynous animals. Kirkpatrick & Ryan, 1991.

### 2.2.1 Population Genetics

Population Genetics describes evolution in terms of changes in genotype frequencies. Kirkpatrick (1982) considers two haploid gene loci; the female preference locus  $P$  with alleles  $P_0$  (no preference) and  $P_1$  (preference for a male ornament), the male ornament locus  $T$  with alleles  $T_0$  (no ornament) and  $T_1$  (having the ornament).  $p$  and  $t$  are the relative allele frequencies of  $P_1$  and  $T_1$ .  $D$  is the linkage disequilibrium parameter, describing the association between  $P_1$  and  $T_1$ . The change in  $p$  and  $t$  from one generation to the next is described by equations (1) and (2). Where  $A$  described the net effect of selection on the trait, a function of  $p$  and  $t$ .

$$\frac{dt}{d\tau} = \frac{1}{2}t(1-t)A, \quad (1)$$

$$\frac{dp}{d\tau} = \frac{1}{2}DA. \quad (2)$$

Therefore  $p$  changes if and only if  $D \neq 0$  i.e. there is an association between  $P_1$  and  $T_1$ . Figure 2a shows that that the system converges to either  $t = 0$  or  $t = 1$ , i.e. loss or fixation of the ornament, or to a line of internal equilibria ( $A = 0$ ) where the costs of the ornament are balanced with the benefits.

### 2.2.2 Quantitative Genetics

Quantitative Genetics describes evolution in terms of changes in average phenotypic values, and to a certain extent takes account of genotypes. Lande (1981) describes the values of a male ornament of size  $t$  and a female preference of intensity  $p$  as autosomally inherited, sex-limited, normally distributed traits with means  $\hat{t}$  and  $\hat{p}$ . The change in these means from one generation to the next are described in equations (3) and (4). Where  $\beta_t$  is the total force of directional selection acting on the ornament,  $G_t$  is the additive genetic variance of the ornament and  $G_{tp}$  is the additive genetic covariance between the ornament and the preference.

$$\frac{d\hat{t}}{d\tau} = \frac{1}{2}G_t\beta_t, \quad (3)$$

$$\frac{d\hat{p}}{d\tau} = \frac{1}{2}G_{tp}\beta_t. \quad (4)$$

The line of equilibria corresponds to when  $\beta_t = 0$  i.e. where natural and sexual selection are balanced. Figure 2b shows this line is stable when the slope is larger than  $G_{tp}/G_t$ . If  $G_{tp}$  is very large the line can be unstable.

### 2.2.3 Invasion Analysis

Invasion Analysis looks at the dynamics of a monomorphic population invaded by a rare mutant, seeing where the mutant trait succeeds to substitute itself as the populations resident trait. Pen and Weissing (2000) considered a population consisting of both non-ornamented and ornamented males where the ornamented males have a reduced viability of a factor  $1 - s$ . Females have a preference  $p$  for mating with an ornamented male and the male has a tendency  $t$  to develop the ornament.  $W(t, p|t^*, p^*)$  is the fitness of a rare mutant of type  $(t, p)$  in a resident population of type  $(t^*, p^*)$ , this is derived systematically from life-history considerations. The total derivatives of  $W$  with respect to  $t$  and  $p$  at  $(t^*, p^*)$  are shown in equations (5) and (6).  $v_{m1}$  and  $v_{m0}$  are the reproductive values of ornamented and non-ornamented males in the resident population.  $b_{pt}$  describes the statistical association between the trait and the preference.

$$\frac{dW}{dt} = (1 - s)v_{m1} - v_{m0}, \quad (5)$$

$$\frac{dW}{dp} = b_{pt}((1 - s)v_{m1} - v_{m0}). \quad (6)$$

At steady state ( $\frac{dW}{dt} = \frac{dW}{dp} = 0$ ) we have that  $(1 - s)v_{m1} = v_{m0}$  i.e. the costs of the ornament are equal to the benefits. There is a line of equilibria, shown in Figure 2c, the approach of which is governed by the canonical equation of adaptive dynamics (see Dieckmann and Law, 1996).

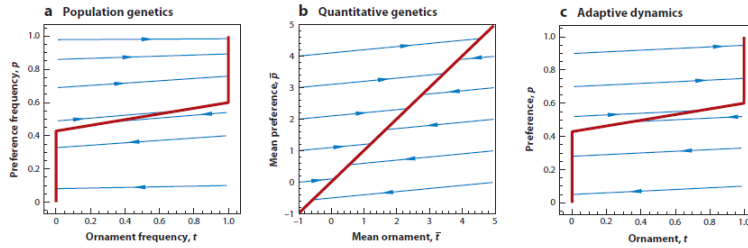


Figure 2: Three implementations of the Fisher process: (a) dynamics of Kirkpatrick's (1982) population genetics model, (b) dynamics of Lande's (1981) quantitative genetics model, (c) adaptive dynamics model by Pen & Weissing (2000). Red lines represent equilibria of the model and blue lines with arrowheads indicate representative trajectories of the dynamical systems. Kuijper, Pen, and Weissing, 2012

### 3 *Drosophila* Courtship Songs

The mating behaviour of *Drosophila* has been extensively studied (see Sturtevant (1915), Bastock & Manning (1955), Manning (1965), Spieth (1974) and Ewing (1983)). During courtship the male brings out one wing and vibrates it rapidly for a few seconds near the female, sending species-specific auditory information to the female. This courtship display can be repeated several times and its success is dependent on both the females receptiveness and the quality of the display. If it is successful, the female will pause long enough for the male to lick the females genitalia and attempt to copulate with her. Copulation can be successful if the female parts her wings. An in-depth description of this courtship behaviour can be found by Bastock & Manning (1955) and Sturtevant (1915).

The compositions of the courtship songs sung in the displays are composed of two elements; the continuous, second-long sine songs and the shorter, millisecond-long repeated pulses (see Figure 3). The pulse components can be characterized by their intrapulse frequency (IPF) and their interpulse interval (IPI).

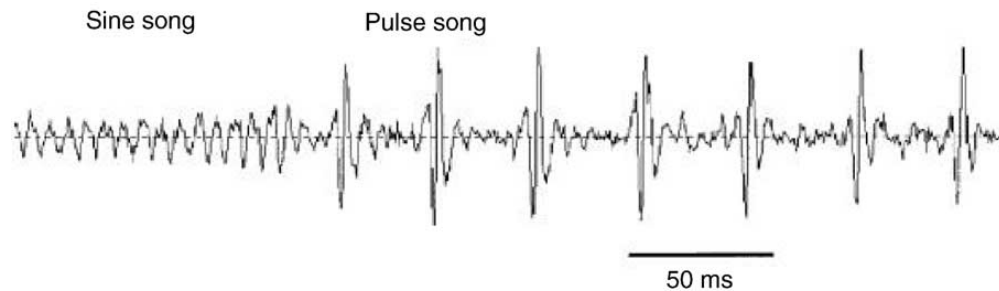


Figure 3: The courtship songs of *Drosophila melanogaster*. Tauber & Eberl, 2003

The courtship songs of different species vary from one another (see Figure 4) and this difference is a crucial component of the flies' premating barrier (Markow & O'Grady, 2005). By studying the songs and antennal tuning of seven members of the *melanogaster* species group Raibinina *et al* (2011) showed *Drosophila* antennal ears are mechanically tuned to different best frequencies and furthermore that these frequencies correlate with the high frequency pulses of the conspecific courtship songs.

#### 3.1 Actively Tuned Ears

A laser Doppler vibrometer was used by Raibinina *et al* (2011) to measure the flies antennal ear movement without stimulation. The free fluctuations found represent both the passive motion due to surrounding air particles and the active motion due to the auditor neurons mechanical feedback. The flies can be CO<sub>2</sub> anesthsised to eliminate the active motion effect. The active mechanical feedback shifts the sound receivers best frequency from around 800Hz to below 200Hz, representing the frequency range of the male sound emissions. Experimentally it was found that this frequency tuning of the flies ears was strongly correlated with the highest frequency pulses occurring in the conspecific songs (see Figure 5). Thus the flies frequency tuning is dependent on active mechanical feedback.



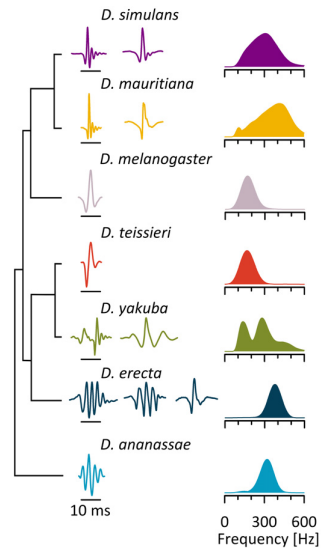


Figure 4: Composition of pulses for different *Drosophila* species. Cladogram of species used (left), time traces for pulse types (middle), spectral composition of the pulse type with the highest IPF (right). Riabinina *et al*, 2011.

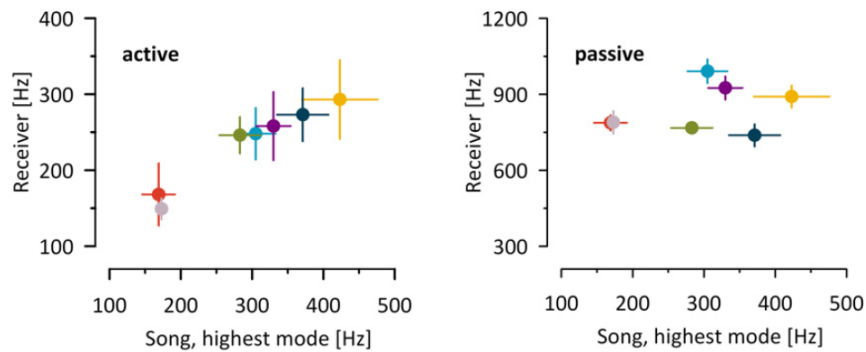


Figure 5: Receiver tuning against the highest frequency pulses of the conspecific courtship songs in the active and passive receivers of flies. Riabinina *et al*, 2011

## 4 Variation in Female Choosiness

We wanted to test whether there was any variation in the choosiness of females in different species of *Drosophila*, i.e. if there is any variation, inter & intraspecifically, in how much the female cares about the mating song being at a particular frequency.

Our initial idea to test for variation in choosiness was to play a series of pre-recorded pulse components of the conspecific song to a female and muted male couple, changing the frequency of the pulses each time. We would repeat this with three different species and quantify the degree of choosiness by the time taken to copulation.

### 4.1 Implementation

We used recently eclosed virgins from the species *Drosophila melanogaster*, *Drosophila tessieri* and *Drosophila yakuba*, and clipped the males wings. We wanted to do each experiment at the same peak activity times, so the flies were trained to different circadian rhythms beforehand for experimental convenience. The activity peak for *Drosophila melanogaster* is shortly after dark (see Figure 6), so we had one set of flies in light from 00:00-12:00, another from 02:00-14:00 and another in light from 04:00-16:00.

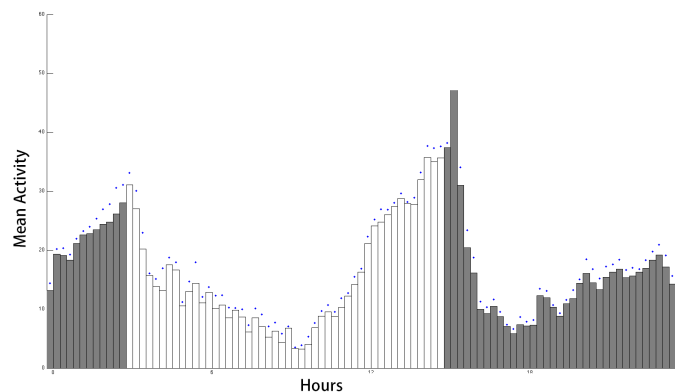


Figure 6: Average Activity During Entrainment in Canton S, unpublished graph provided by Joerg Albert.

We played the songs to the couple via a shaker directly underneath the mating chamber. In theory, the shaker should vibrate the air particles near the females ears in the same way as if the male was naturally singing to her. The songs were played at  $-12.5\text{dB}$  and the distance of the chamber from the shaker was kept the same each time. A small amount of food was put in the chamber and the male and female were kept separate until the experiment began.

### 4.2 Results

We first used *Drosophila melanogaster* Canton S couples. The couples were put together for 10 minutes in silence and then the song was played to them for a further 10 minutes. The first experiment was to check everything was working OK, and the results were promising.

Although the couple never mated, they appeared to be a lot more active when the song was played and went through a lot of the courting routines. However, we couldnt replicate this experiment (playing the song made no obvious difference to the behaviour of other Canton S couples we looked at) and thus felt that there was something wrong with the experimental set-up.

### 4.3 Improvements/ Future Technique

The mating chamber only had a sound-permeable floor, so the sound could have been echoing around in an unnatural and distracting way. We considered changing the ceiling of the chamber to one made from the same mesh as the floor; however this would greatly impair the view of the courtship and hence make analysis of the behaviour very difficult. To run this experiment more successfully an idea would be to use an electrode to send the frequencies of the song to the couple rather than by using the shaker. The couple would be put on a metal courting platform with an electrode inserted through the middle (and insulated from the platform). The electrical pulses should reach all areas of the plate, and the difference in intensity should be negligible throughout the plate. This would work in a similar way (but from a further distance) to the method used by Raibinina *et al* (2011) to investigate antennal movement. It would hopefully eliminate the echoing problem we had, and thus make the song effect more realistic.

## 5 Tracking Flies

There are several types of female movement during courtship; standing still and listening to the male, walking independently of the what the male is doing (and the male either following her, or independently moving) or being chased by the male. Hence being able to track a courting *Drosophila* couple in an accurate and consistent way would be helpful when trying to analyse female behaviour.

Existing programs for tracking often fail when two flies get too close to one another (Branson *et al*, 2009), which is clearly a major problem when tracking courtship. For the resolution we need to accurately analyse the flies wing movement when “singing” (for cross analysis with the audio recordings) the video needs to be taken from close up (see Figure 7a). Branson *et al* (2009) developed a machine-vision algorithm to track flies with this issue in mind and successfully managed to keep the tracking identities for each fly, however the videos we have from audio recording videos are not suitable as an input for this program as they are too close up (see Figure 7b).

Two tracking techniques in Mathematica were developed to begin to try and solve the problem of tracking flies from close-up videos<sup>2</sup>. An existing video<sup>3</sup> of a courting Oregon R strain of *Drosophila melanogaster* male and female (with her wings clipped) was used to implement an ImageFeatureTrack technique. A pair of Canton S *Drosophila melanogaster* flies (not in the process of courting) were filmed<sup>4</sup> for use with an ImageValuePositions technique.

<sup>2</sup>If you would like a copy of any of the videos mentioned in this report please contact Chris Langridge (c.langridge@ucl.ac.uk)

<sup>3</sup>OregonR\_11.6\_12.007-1.avi

<sup>4</sup>Exp\_0025-1.avi

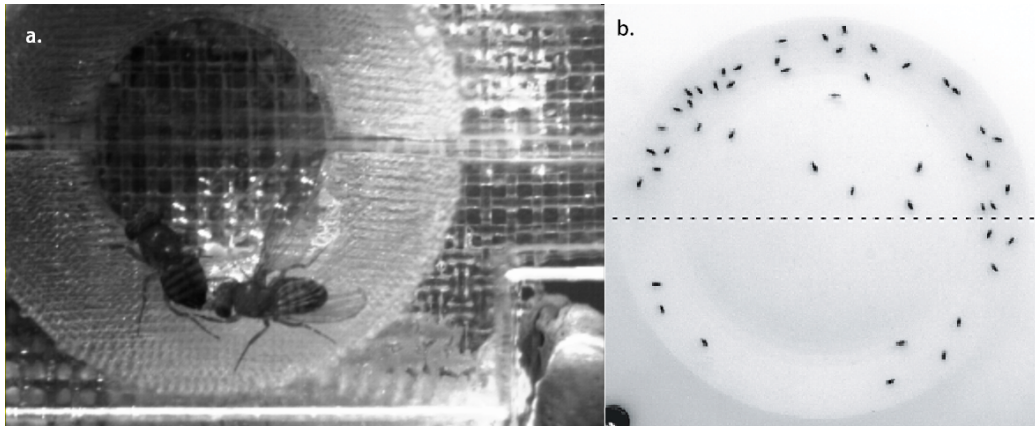


Figure 7: (a) An example of a frame of a video taken when recording audio, two flies in a 1cm diameter chamber. (b) An example of a Ctrax video input frame, 50 flies in a 12.7cm in diameter chamber, from Simon & Dickinson (2010)

## 5.1 ImageFeatureTrack Technique

Due to the time limitations of this project we first used previously recorded videos of courting flies. The videos were taken in an experiment to record the different courtship songs of a variety of species and therefore were not filmed with video analysis in mind.

The ImageFeatureTrack function in Mathematica was implemented (see Appendix A), this tracks feature points in a sequence of images. Initial coordinates of (102, 130) were used for the female and (161, 134) for the male (both taken as an estimated midpoint of their thorax), tolerance was set of 10 and a maximum of two features were tracked. Figure 8a shows some frames from the first 660 frames of the video where the tracking was good, and Figure 8b shows some frames from where the tracking broke down. It proved difficult to track one point on the fly accurately throughout the video, the point would sometimes hover at different points on the flies body, or switch from fly to fly<sup>5</sup>.

### 5.1.1 Results

The video tracking broke down after about 660 frames, we assumed the cause of this was the fact that the flies went into the shadowed area at the top of the frame. The speed of the female and male was found for the first 660 frames and then fitted to a 40<sup>th</sup> degree polynomial for a clearer analysis (see Figure 9a). It is clear from the graph that the male and female roughly have the same speed throughout the 660 frames, which can be seen in the video. The male tends to accelerate just before the female does, which could reflect the male pushing motion seen in the video. The distance between the male and female seems to decrease at points of increased/ fluctuating acceleration, which could correspond to increases in the males forcefulness. Figure 9b shows the trajectories of the tracking points.

Obviously this tracking technique is very flawed, but could have been improved somewhat with more contrast between the flies and the background. Perhaps by using a white background and better lighting.

<sup>5</sup>see IFT\_tracked\_OregonR\_11.6#79CA060.mov for the tracked video.

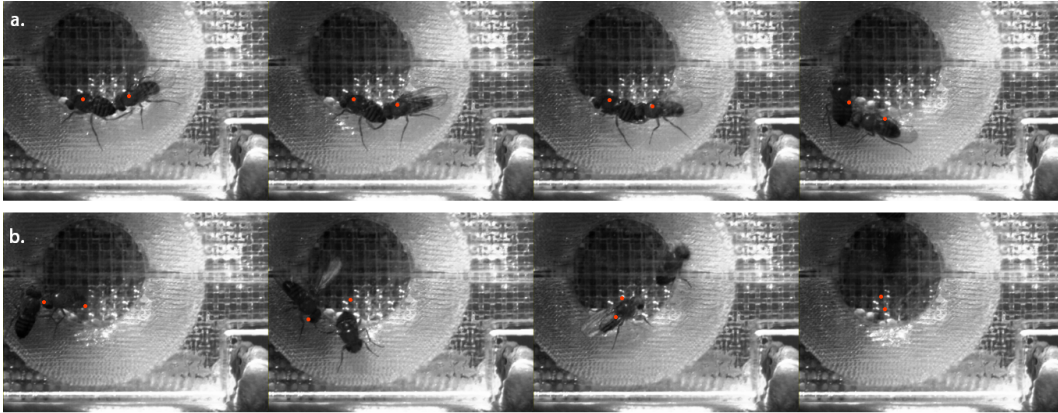


Figure 8: Tracking use the ImageFeatureTrack technique. (a) Frames 1, 100, 300 & 500 where the tracking works. (b) Frames 690, 810, 870 & 960 where the tracking breaks down.

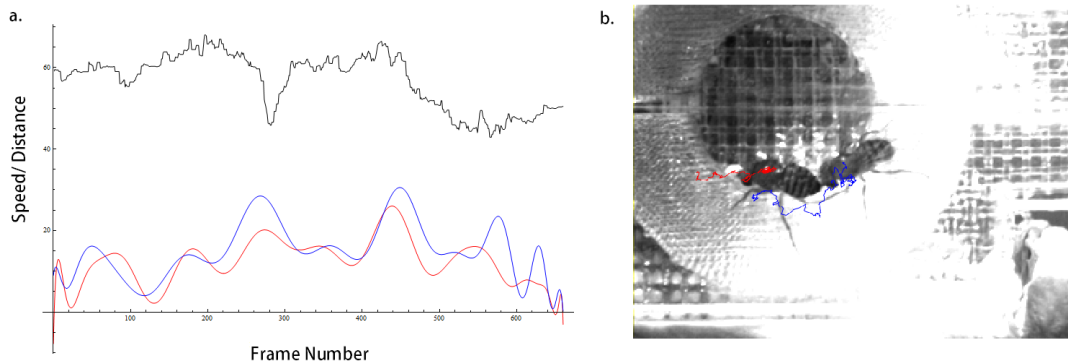


Figure 9: Using ImageFeatureTrack technique. (a) The distance between the flies (black) and the fitted speed of both the female fly (red) and the male (blue) for the first 660 frames of the video. (b) Tracking movements for both the female (red) and the male (blue) during the 660 frames overlaid onto the first frame.

## 5.2 ImageValuePositions Technique

It is possible to find the coordinates of *Drosophila* eyes from a colour video frame as the contrast between the red of their eyes and the rest of the image is sufficiently large. This fact was used to develop another type of tracking.

First a video frame is binarised where any pixel in which the red component is  $> 0.59$  and  $< 0.85$  and the green component is  $< 0.2$  is converted to a white pixel, and the rest of the image is converted to black (see Figure 10a). Then using this binarised image the coordinates of the white pixels (i.e. the eyes) are found using the ImageValuePositions Mathematica function. These coordinates are then clustered into two groups of similar coordinates, and the mean is taken for each cluster. This mean coordinate is used as the tracking point, shown in Figure 10b. See Appendix B for the code used to implement this technique.

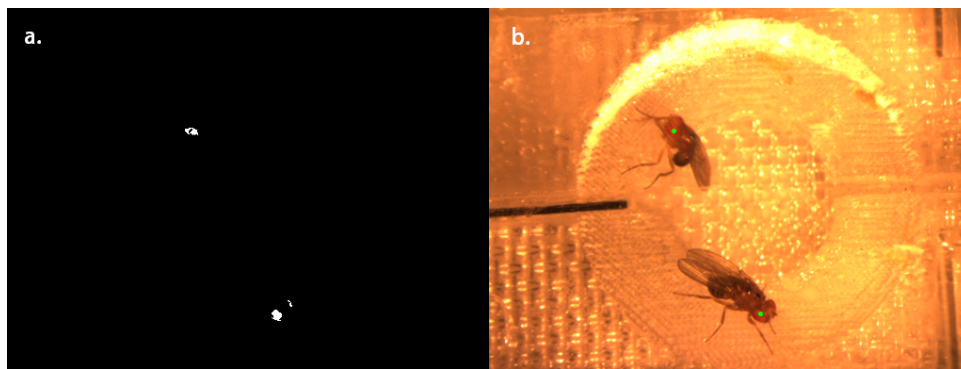


Figure 10: Tracking by the ImageValuePositions technique. (a) The red points are converted to white and everything else black, and small groups of pixels are deleted. (b) Two clusters of white coordinates are identified and the mean coordinate for each is taken and marked with a point (for clarity the points here are marked in green, however in the actual tracking they are marked in red).

### 5.2.1 Results

This technique was applied to the first 1000 frames of the Exp\_0025-1 video<sup>6</sup>. The speed of both the flies and the distance between them was found (see Figure 11a). The tracking was quite good in comparison to the ImageFeatureTrack technique.

Problems occurred when the flies' heads got too close and then the tracking points could swap over from one fly to the other (Figure 12a) and at some points both the tracking points could both be on one fly (Figure 12c). These issues are caused by the fact that the mean coordinates are taken from the two biggest red clusters, so there are cases where both eyes of one fly and one eye of the other is taken as one cluster (Figure 12 a & b), and then the mean is seen floating in the space between. This problem could be overcome if it was possible to mark one fly in one colour and the other in another.

<sup>6</sup>see IVP\_tracked\_Exp\_0025-1.mov for the tracked video.

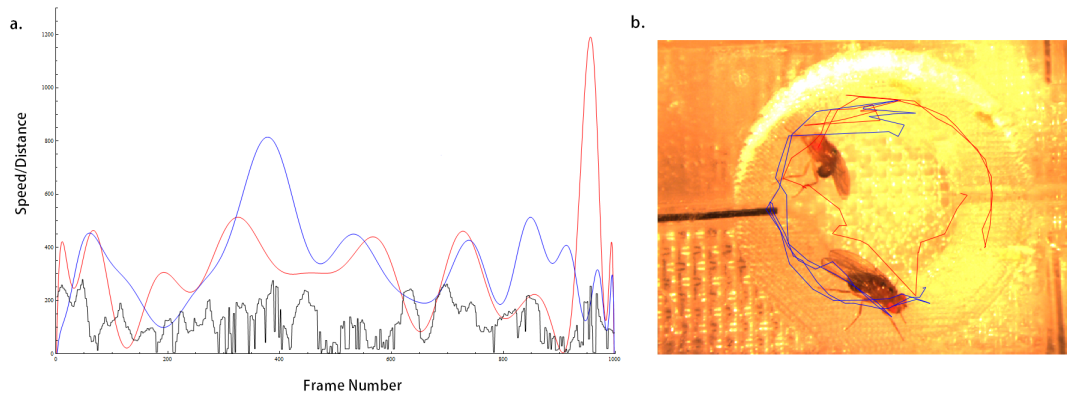


Figure 11: Using ImageValuePositions technique. (a) The distance between the flies (black) and the fitted speed of both fly 1 (red) and fly 2 (blue) for the first 999 frames of the video. (b) Tracking movements for both fly 1 (red) and fly 2 (blue) during the first 200 frames overlaid onto the first frame.

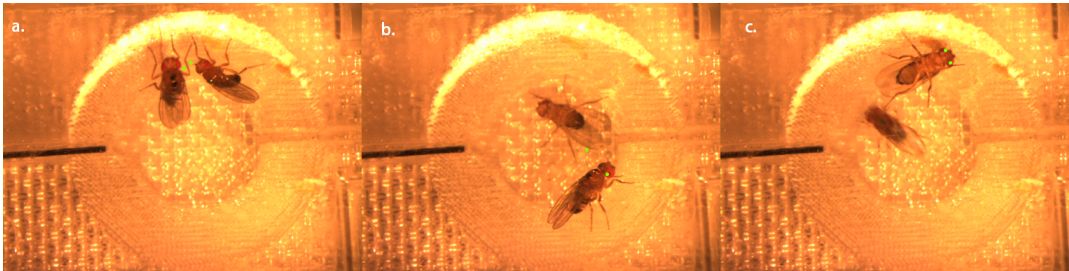


Figure 12: Frames where averaging did not provide accurate results. (a) Frame 75, (b) Frame 215 & (c) Frame 356.

### 5.3 Limitations

Apart from the tracking problems already mentioned, both techniques took quite a while to run. The 660 frames analysed using the ImageFeatureTrack technique took about 6 minutes, and the 1000 frames analysed using the ImageValuePositions technique took about 20 minutes.

Furthermore both techniques require someone to manually either find the initial coordinates of the fly (easily done in Mathematica) or to set the pixel colour boundaries for what colour has been selected to track (which can be a bit more difficult and time consuming).

Hence these approaches are not ideal for the analysis of many long videos (which is what would be needed for significant results of behaviour).

## 5.4 Future Technique

Being able to track a set of two coloured-on dots on the fly's thorax would allow us to track its orientation as well as its movement. A variety of substances were experimented with to make these dots, but none tracked very well due to a weak contrast in colour between them and the flies body (coloured markers); leaking into/ spreading around the flies body (UV solution); or too much similarity between them and reflections from the mesh flooring (correcting fluid). Hence a recording of flies with two small dots with enhanced colouring (perhaps by using a quickly drying UV solution) on their backs and using the ImageValuePositions technique could work quite well in tracking the flies' movements accurately.

Being able to track four differently coloured points, two on each fly, would be ideal as we would then be able to work out direction of travel, orientation and amount of movement. This information would be extremely interesting when studying courtship interactions. For example, it would allow us to find the angle the female positions herself in in order to hear the male song optimally.

## 6 Discussion

The behavioural response of female *Drosophila* to courtship from males is well reported, as are the acoustic details of courtship songs and the mechanisms of the *Drosophila* antennal ears. This report has provided preliminary development of a tracking technique which could be used to produce results for a more in-depth analysis of female responses to courting songs.

Further work with the aid of such a tracking technique could include investigating degrees of female choosiness. Basic results from tracking courting flies (such as time spent running away from a male or standing still listening to a male) could be used to quantify individual choosiness in females. The future experimental technique and ideas suggested in Section 4 could be used in this investigation. It would be interesting from an evolutionary perspective to use such results to see if there is any variation in female choosiness in different *Drosophila* species.

Another interesting outcome of a successful tracking technique is its use in setting realistic frequency parameters for antennal observations. The amount of passive antennal displacement (by air particles bombardment whilst the fly is moving) could be well estimated for different types of movement by finding the speed. It would then be possible to measure antennal displacement from sounds including both courtship elements and realistic elements corresponding to fly movement when investigating how the ears of *Drosophila* work under more life-like conditions.

Applying a successful tracking technique to high resolution videos captured when courtship audio is being simultaneously recorded, could yield accurate and consistent results in how exactly females respond to specific parts of courting songs. This report has developed some ideas for tracking such videos. With further work, specifically on the technique used to mark flies with colours, this tracking technique might not be far off.



# Appendix

## A. ImageFeatureTrack Mathematica code

### ImageFeatureTrack technique

Import video:

```
f[x_] :=
  Import[
    "/Network/Servers/xgrid.complex.ucl.ac.uk/Volumes/Users/elizabethgallagher/Desktop/CP1/OregonR_
    11_6_12_007-1.avi", {"Frames", x}]
```

Half the size of frame for quicker computing:

```
f2[x_] := ImageResize[f[x], Scaled[1/2]]
```

Implement ImageFeatureTrack, and highlight these coordinates on the original frames.

```
y = ImageFeatureTrack[Map[f2, Range[660]], {{102, 130}, {161, 134}}, Tolerance -> 10,
  MaxFeatures -> 2];
track = Map[HighlightImage[f2[#], y[[#]]] &, Range[660]];
```

Visualise movement:

```
ListLinePlot[Transpose@y, PlotRange -> {{0, 200}, {0, 200}}]
```

Distance between the flies in each frame:

```
d = Map[EuclideanDistance[y[[#]][1], y[[#]][2]] &, Range[660]];
```

Find speed in distance unit per second (filmed at 30 frames/sec.):

```
sf = Map[EuclideanDistance[y[[# + 1]][1], y[[#]][1]] / (1/30) &, Range[659]];
sm = Map[EuclideanDistance[y[[# + 1]][2], y[[#]][2]] / (1/30) &, Range[659]];
```

Find fits for these:

```
fitf = Fit[sf, Map[x^# &, Range[0, 40]], x];
fitm = Fit[sm, Map[x^# &, Range[0, 40]], x];
```

Plot distance and speed:

```
Show[Plot[{fitf, fitm}, {x, 0, 660}, PlotLegends -> {"Female Speed", "Male Speed"},
  PlotRange -> {{0, 660}, {0, 70}}, AxesLabel -> {"Frame Number", "Speed/Distance"},
  PlotStyle -> {Red, Blue}], ListLinePlot[{d}, PlotLegends -> {"Distance between female and male"},
  PlotStyle -> Black, PlotRange -> All]]
```

Export video with tracking points highlighted:

```
Export["track3_OregonR_11_6_12_007-1.mov", track, "FrameRate" -> 30];
```

## B. ImageValuePositions Mathematica code

### ImageValuePositions technique

Import file and half the size for quicker computation:

```
g[x_] :=
  Import[
    "/Network/Servers/xgrid.complex.ucl.ac.uk/Volumes/Users/elizabethgallagher/Desktop/CP1/Exp_0025-1.avi", {"Frames", x}]

g2[x_] := ImageResize[g[x], Scaled[1/2]]
```

Find coordinates of red points, cluster into 2 sets and find the mean for each set:

```
g3[x_] := DeleteSmallComponents[Binarize[g2[x], #[1] > 0.59 && #[1] < 0.85 && #[2] < 0.2 &], 4]

g4[x_] := With[{c = ImageValuePositions[g3[x], White]},
  With[{d = FindClusters[c, 2]}, Map[Table[Mean[Map#[[i] &, d[[#]]], {i, 2}] &, Range[Length[d]]]]]
```

Highlight tracked points on original frames:

```
y = Map[g4[#] &, Range[1000]];
track = Map[HighlightImage[g2[#], y[[#]]] &, Range[1000]];
```

Export video with tracking points highlighted:

```
Export["track_Exp_0025-1.mov", track, "FrameRate" -> 10]
track_Exp_0025-1.mov
```

Visualise movement:

```
ListLinePlot[Transpose@y, PlotRange -> {{100, 500}, {0, 400}}, Axes -> False]
```

Distance between the flies in each frame:

```
e = Map[EuclideanDistance[y[[#]][1], y[[#]][2]] &, Range[660]];
```

Find speed in distance unit per second:

```
f1 = Map[EuclideanDistance[y[[# + 1]][1], y[[#]][1]] / (1/10) &, Range[999]];
f2 = Map[EuclideanDistance[y[[# + 1]][2], y[[#]][2]] / (1/10) &, Range[999]];
```

Find fits for these:

```
fitf1 = Fit[f1, Map[x^# &, Range[0, 40]], x];
fitf2 = Fit[f2, Map[x^# &, Range[0, 40]], x];
```

Plot distance and speed:

```
Show[Plot[{fitf1, fitf2}, {x, 0, 49}, PlotLegends -> {"Fly 1 Speed", "Fly 2 Speed"},
  PlotRange -> All, AxesLabel -> {"Frame Number", "Speed/Distance"}, PlotStyle -> {Red, Blue}],
  ListLinePlot[{e}, PlotLegends -> {"Distance between fly 1 and fly 2"}, PlotStyle -> Black,
  PlotRange -> All]]
```

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