DIFFERENCES IN VELOCITY BETWEEN THE SEXES AND STAGES OF COURTSHIP IN A DANCE FLY

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Male and female *Hilara maura,* photo by: Freddie Hunter

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Abstract

Dance flies (Diptera: Empididae: Empidinae) are famous for their elaborate mating behaviour, which can be broken down to three distinct stages in adults: hunting, swarming, and mating. In many species, males hunt for prey that they present as nuptial gifts to recipient females in swarms, after which the pair will copulate in the air or after landing on nearby vegetation. Dance flies are also famous for the female sexual ornaments that occur in approximately a third of the species in the family, which challenges theories on the nature of ornament evolution. The ornamentation can take the form of feathery legs, inflated abdomen, or enlarged or darkened wings, and are mostly theorized to alter their silhouette to make them appear larger. The progress in studying mating systems in dance flies has been hampered by the fact that dance flies do not behave as expected in lab environments and by the speeds of natural swarms. Some prior hypotheses for sexual selection include scramble contest where the fastest individuals gain access to the opposite sex and mate choice where individual velocity is a preferred trait. The differences in velocity could also be due to load lifting constraints caused by the extra weight of the caught prey the males carry or as a response to predation avoidance or hunting success. In this thesis I use high speed video and tracking software to observe and quantify sexual differences in swarming behaviour including the flight paths and velocities of the sexes at different stages of courtship (hunting, swarming, and mating). I showcase the possibility of using high speed video when studying dance flies and I show that swarming males fly slightly faster than swarming females, and hunting males fly slightly slower than swarming females. The fact that hunting males are slower than the swarming participants is inconsistent with the hypothesis that the velocity differences are based on hunting success or predation avoidance, but wholly consistent with sexual selection.

Keywords: Dance fly, *Hilara maura*, high-speed camera, mate choice

Abstract (Svenska)

Dansflugor (Diptera: Empididae: Empidinae) är kända för deras välutvecklade parningslek, som i fullvuxna individer kan bli nedbruten i tre distinkta faser: jakt, svärmning, och parning. Hos många arter jagar hanar efter byten som de sedan presenterar som bröllopsgåvor till mottagliga honor. Efteråt kommer paret para sig i luften eller på närliggande vegetation. Dansflugor är också kända för könsspecifika ornament som visar sig hos honor i ungefär en tredjedel av arterna i familjen, dessa utmanar teorier om ornamentevolution. Ornamenten kan vara håriga ben, uppblåsta magsäckar, eller förstorade eller mörknade vingar. Syftet med ornamenten tros vara att ett sätt för honorna att se större ut, kanske för att anses som mer fertila. Forskningen av parningssystemet har hållits tillbaka av svårigheterna att följa snabba svärmar samt att dansflugor inte går att studera in vitro. I tidigare hypoteser för sexuell selektion ingår scramble-konkurrens, där de snabbaste individerna får tillgång till det motsatta könet samt partner-val där individens hastighet är en önskad egenskap. Hastighetsskillnaderna kan också bero på den extra tyngden hanarna bär på i form av det fångade bytet eller som en respons till predation eller jaktlycka. Jag använder en höghastighetskamera och mjukvara för spårning för att observera och kvantifiera sexuella olikheter i svärmningsbeteende som inkluderar flygmönster och hastigheter för de olika könen och stadierna av uppvaktning (jagande, svärmande, parning). Jag påvisar möjligheterna av användandet av höghastighetskamera för forskning av dansflugor och jag visar att svärmande hanar är något snabbare än svärmande honor samt att jagande hanar är långsammare än svärmande honor. Faktumet att jagande hanar är den långsammaste gruppen är oförenlig med hypotesen om jaktlycka eller predationsavvikande men är mer konsekvent med teorier i sexuell selektion.

Nyckelord: Dansfluga, *Hilara maura*, höghastighetskamera, partner-val

Introduction

Evolution has found many ways for organisms to spread their genes by sexual reproduction, creating an incredible diversity in nature, not only in animal appearance but also in behavior. Many species exhibit sexual dimorphism and more often than not the sex to invest in ornaments or performance traits, that are used to attract mates, are males, with some of the most famous examples coming from birds (Andersson, 1982). Another sexual dimorphism that are used for the access of opposite sex gametes by intrasexual competition is the formation of antlers in many species (Clutton-Brock, 1982). Sexual dimorphism can also be targeted at physiological performance traits such as strength or locomotion (Lailvaux et al., 2003). Even so, antler size in deer, ornamentation among birds, and differences in physiological performance vary enormously and the origin of these elaborate sexually dimorphic traits are well documented in some species while in others, such as the dance flies, the cause is very much still a mystery, and the diversity in the family is hard to explain. One subfamily, Empidinae, exhibits similar courtship behaviors across the taxa, but approximately only a third of its species also show varying levels of female ornamentation (Cumming, 1994). Mating behavior has been notoriously hard to study in dance flies, with key constraints being their unwillingness to behave in lab environments and the difficulty to follow individual participants in large and fast-moving wild mating swarms, and even less is known about the intersexual differences in performance traits.

Operational sex ratio

One important metric that can help explain the differences and the diversity in mating systems, and therefore also intrasexual mate competition and mate choice, is the operational sex ratio (OSR). OSR has been defined as the ratio of males to females that are ready to mate in a given time and space (Emlen & Oring, 1977; Kvarnemo & Ahnesjo, 1996). Mate choice is usually more common in the sex that OSR is skewed against. For example, if there are more males than females ready to mate at a given time and space then the males are expected to compete for females and the females are expected to exhibit more mate choice than males by discriminating among mates. In other words, it is less costly to be choosy if the members of the opposite sex are abundant.

A broad definition of mate choice is when a trait in one sex leads to non-random mating decisions by the opposite sex (Edward, 2014). This trait can be a behavioral one, such as flying velocity, or a secondary sex characteristic, such as an ornament. Elaborate ornaments, such as long feathers, have evolved in many species since they are preferred by the opposite sex. Females do usually have a larger parental investment than males thanks to their investment in larger gametes. In cases where parental investment is larger for one sex the opposite sex will usually compete among themselves for mating opportunities with that sex. Therefore females are usually the sex that exhibit mate choice (Trivers, 1972).

Importance of intersexual velocity differences

The physiological differences between the sexes can be an indication of mate choice, for example, Sullivan (1981) generated a number of hypothesis for the relative speeds of swarming insects, and some of these relate specifically to sexual selection. One hypothesis being that males in swarming species will exhibit faster average speeds inside swarms than female because of female mate choice. The differences in physiological traits can also be attributed to scramble competition. In scramble competition participants compete over a finite resource and a first-come, first-served principle is in place. In scramble mating

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competition, no mate choice needs to be active and in a species where males approach females the male that finds and approaches a female first can be the one that copulates irrespective of mate choice (Alcock, 1980). In a scramble mating competition, faster would therefore be better. One might also predict a higher variance in the performance trait within males if scramble competition was true since males would have two modes of swarming, patrolling in the search for a female, and accelerating when they notice and try to approach the female.

Dance fly

The dance flies (sometimes called balloon flies or dagger flies) from the subfamily Empidinae (Diptera: Empididae) is divided in to three genera, *Hilara*, *Empis* and *Rhamphomyia* with approximately 1,450 species worldwide (Chvála, 1976). Empidinae are known for their unusual mating behavior where individuals aggregate in mating swarms (with the exception of a few species (Chvála, 1976)) and where the males approach the females from below (Downes, 1970). This has caused the male eye facets in many species to be enlarged dorsally (Chvála, 1976; Svensson, 1997). In these swarms the male presents the female with a nuptial gift, often in the form of prey, sometimes wrapped in silk (Eltringham, 1928). The nuptial gift is the only way most adult females in Empidinae species get their protein (Downes, 1970). Hunting males are believed to experience higher mortality rates than the more stationary females (Svensson & Petersson, 1987; Thornhill, 1979).

Some species of Empidinae have elaborate ornaments that are only expressed in females (Cumming, 1994). In approximately 28% of the species in the genera *Rhamphomyia* and *Empis*, females have some sort of secondary sexual characteristic (Cumming, 1994). These ornaments include inflatable abdomens, pinnate (feathery) leg scales and enlarged or darkened wings. The female ornaments are believed to be a signaling mechanism to attract mates (Cumming, 1994), either for their gametes or to obtain more prey from males to provide for ovarian maturation since the mating behavior is repeated by both sexes. (Downes, 1970; Hunter & Bussière, 2019; Svensson & Petersson, 1987). Intriguingly, some of these female signals are believed to be deceptive, with examples of both inflated abdomen and pennate leg scales that enlarge the female silhouette in mating swarms, although studies of related dance fly species have shown a correlation between female size and fecundity (Funk & Tallamy, 2000; Hockham & Ritchie, 2000; Svensson & Petersson, 1987). A possible answer for the diversity in female ornamentation in dance flies might have to do with the trade-offs ornaments have with other life history traits such as survival or fecundity. Females that invest in secondary sexual traits do so by allocating resources from one life history trait to another and therefore they might lose out on their own fecundity (Fitzpatrick et al., 1995). Studies have also shown increased predation risk in female dance flies with ornaments compared to their ornament-less male counterparts (Gwynne & Bussière, 2002; Gwynne et al., 2007). Females are not the only ones being deceptive however, there are species where males try to present a nuptial gift that is without nutritional value, even in a species where the female is believed to be dependent on the protein source the male presents for contribution to female ovarian development (Preston-Mafham, 1999). The deceptiveness of the female ornamentation could lead to sexual antagonistic co-evolution where males that go for the most ornamented female has a lower chance for paternity thanks to the abundance of mates the popular females get (Herridge et al., 2016).

There are some species of Empidinae where the gift presented has low to no nutritional value (as a rule, in contrast to the cheating males in the previous example) (Kessel, 1955) and

therefore balloon size (the size of the wrapped gift) could be a cue representing male genetic quality rather than a source of protein (Sadowski et al., 1999). The evolution of "worthless" gifts is believed to stem from previously nutritious nuptial gifts (LeBas & Hockham, 2005). The low nutritional value from the gift can have developed the females in these species to mature their eggs without the need of adult dietary protein (Cumming, 1994), and where they no longer need external nutrients supplied by a gift.

It is easy to link mate choice and female ornamentation to sexual selection. The definition of sexual selection, and how to measure it, has since Darwin named it however been a topic of discussion (Kokko et al., 2012; Shuker & Kvarnemo, 2021). However, the ornamentation in female dance flies does not necessarily have to come from sexual selection. If the mating behavior in females is based on resource gathering rather than gamete assessment, then the ornamentation is a response to natural selection rather than a sexual one. The definition might not matter in this thesis since the methods of measuring sexual selection can also be used to measure mate choice.

An interesting aspect of presenting prey and mating in flight is the inevitable effect of the load lifting constraint males in swarms will experience, small as it might be. The discussion (Marden, 1989) has mostly focused on the weight the male can carry and how it could affect attracting larger, more attractive, females. A male in a swarm will carry a prey and a male in mating will carry both the prey and their mate. This taken in isolation would cause mating pairs to be significantly slower that swarming males and swarming males significantly slower than hunting males and swarming females. Still, in one species of dance fly, a previous study has shown that the load lift had no negative impact on male mating success (Murray et al., 2019).

Several lines of evidence suggest that males are choosy when it comes to mate assessment in many Empidinae. Males monopolize the protein source in many species (Svensson & Petersson, 1987), which creates an incentive for females to compete for males, as female fitness probably increases with multiple matings (Arnqvist & Nilsson, 2000). More so, males invest largely in reproduction by increased predation risk and time spent on finding prey since they are the only sex that hunts (Svensson & Petersson, 1995). The OSR varies between species and time of swarming period and where males are usually scarcer later in the swarming season (Svensson & Petersson, 1987, 1995). Some studies have shown that males prefer certain female attributes (Funk & Tallamy, 2000; LeBas et al., 2003; Murray et al., 2018; Svensson & Petersson, 1988; Svensson et al., 1989).

Hilara maura

Hilara Meigen is a large genus that is part of the Hilarini-tribe. Species in Hilarini are frequently sexually dimorphic, and a basal feature is a swollen prothoracic basitarsus (leg segment) in males that includes a glandular unit that produces silk (Hamm, 1928; Young & Merritt, 2003). The main studied species in this paper is *Hilara maura*. Like other members of the genus, they produce silk even though they often use a remarkably small amount of it when wrapping their prey (Chvala, 1996). It is a common species in most of northern Europe and it both hunts and mates over or near water where it gathers in large aggregations (Chvala, 1996). The hunting swarms can easily be distinguished from mating swarms by differences in flight pattern (author´s own observation, and results below). Mating swarms are usually primarily one-dimensional with the occupants moving back and forth over an imaginary straight line above the water, while the hunting swarms are more irregular, with the males flying in very close

proximity to the water surface and changing direction more frequently than swarming participants, in a patrolling manner looking for prey. The prey consists mostly of chironomids or other Diptera, but other insects have also been observed to be preyed upon (Chvala, 1996). A study carried out over a season in the United Kingdom measured the mean OSR for *H. maura* to be slightly male biased, with 62% of the swarming participants being male (Murray et al., 2020). The sexes have small differences such as size where the males are larger but females do not appear to have ornaments (Chvala, 1996).

Aim

Differences in swarm flight velocity between the sexes in dance flies are unknown, and it is a continuous measure of behaviour that can be extracted from video. Analysing differences among species will require us to quantify differences in swarming behaviour from a variety of species. My work focused on developing methods as well as analysing possible links between flight velocities and mating roles. This paper takes a look at the velocity differences between sexes and the different stages of courtship (hunting, swarming, mating) in the dance fly *H. maura*. The differences in speeds between hunting and swarming participants could be an indication of the selection that takes place between the sexes of dance flies. I propose four different scenarios.

a) Predation avoidance or hunting success. Since males are the only sex that hunts, they might experience more predation pressure away from the safety in numbers that mating swarms provide (Cumming, 1994). Therefore, I expect hunting males to be the fastest group as a result of the increased speed needed when hunting for prey or in avoidance of predation. I also expect the variation in hunting male velocity to be greater than in the other categories since the need for acceleration only happens when speeding towards a prey item or away from a predator and is therefore not constant. If no other force is acting, then we might see swarming males being faster than swarming females because of the added speed swarming males achieved from their genetic make-up from being hunters.

b) Load lifting constraint, where swarming males should experience slower velocities than hunting males and swarming females because of the constraint the added weight the caught prey item causes. Mating pairs should be the slowest group since they carry both the prey item and a female (Marden, 1989). If no other force is acting, then there should be no difference between the swarming participants.

c) Intrasexual competition through scramble competition for females, where swarming males should be the fastest group in their race for mating opportunities (Sullivan, 1981), and where there possibly would be a larger variation of swarming male speeds compared to the other groups since the males would fly at a velocity similar to the other participants and only accelerate when spotting a recipient female.

d) Intrasexual competition through mate choice, where if female choice would be acting as selection on male speed (Sullivan, 1981), we would expect to see swarming males being the fastest group. Likewise, in male choice we would perhaps see males preferring slower females as a cue for fecundity (Bondurianksy, 2001) since there would be a speed constraint acting on the weight of the eggs.
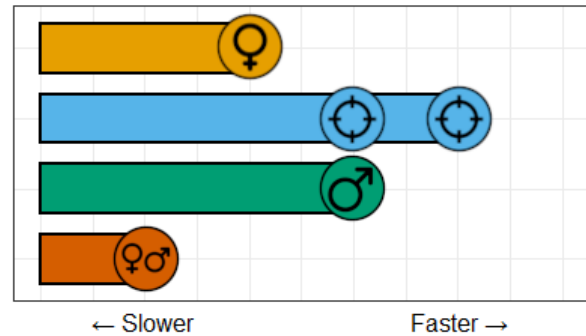
The different scenarios are simplified, and it is possible that more than one of the hypotheses is acting at the same time. A visualisation of the four scenarios is shown in Figure 1.

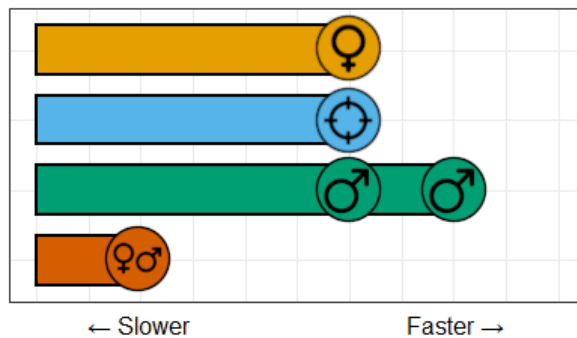**Expected differences in velocities between the sexes and stages of courtship**

Load lifting constraint
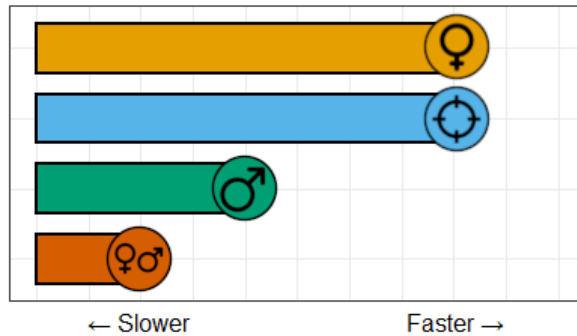
Predation avoidance

Scramble competition

Mate choice

← Slower   Faster →

⚥ Swarming female   ⊕ Hunting male   ♂ Swarming male   ♀♂ Mating pair
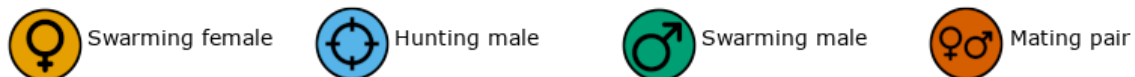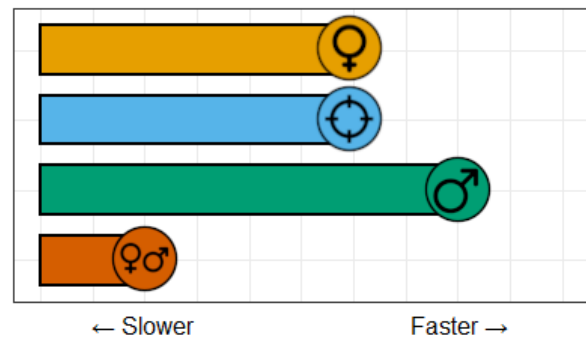
Figure 1 Visualisation of the hypothetical velocity differences between the sexes and stages of courtship and the four different scenarios discussed in the aims. The bars represent average velocity in the groups where larger bars symbolise higher velocity. The multiple symbols in some of the plots represent the two different modes the members of said group shows and would therefore show a larger variation than the other groups. In predation avoidance hunting males fly in a similar pace as the swarming males and when needed they accelerate to a higher velocity. In scramble competition the swarming males have a pace that is similar to the other groups and when spotting a recipient female, they accelerate to a higher velocity to win the race.

Methods and materials

Collection of data

The first set of footage was collected at Sisjön, Göteborg (57°37'28.8"N 11°58'30.2"E), by filming with a Samsung Galaxy s10e mobile phone with the slow motion (240 frames per second (fps)) and super slow-mo (0.4 s of 960 fps or 0.8 s of 480 fps) settings. The footage was filmed in front of a tarp, to provide a contrasting background for easier detection of flies, that was placed between two trees in close proximity behind a swarm of *Rhamphomyia crassirostris*. Unfortunately, the low resolution of these films made analyses difficult (see results). As a consequence, later in the season I arranged access to a professional high-speed MotionBLITZ Eo*Sens*® mini 1 camera with a Nikon 28mm lens provided by VIDIX (http://www.vidix.se/) that was more suitable for filming small moving particles. The camera was operated by using MotionBLITZ Director2 software (https://mikrotron.de/) from a connected laptop. The first of July 2021 between 10pm – 11pm I filmed a swarm of *Hilara maura* at the lake Långsjön in Vallentuna municipality (59°36'45.4"N 18°23'50.2"E) using this

setup. The edge of the swarm was situated around two meters from the lake shore and the swarms was approximately 5 m in diameter and situated around 10 cm above the surface of the lake. I used three 850 nm IR-lights with 30° light angle to illuminate the swarm without disturbing the flies (S-SA6P-IR PoE IR Illuminator, model 20100298). The camera was situated on a small tripod as close to the water surface level as possible, in this case approximately 20 cm. Besides *H. maura* the swarm also contained water striders (Gerridae) and caddisflies (Hemiptera), but these were easily differentiated in the footage because of difference in their size and behaviour relative to *H. maura*.

Filmed material

Regardless of the filming speed, the camera produces 1636 8-bit grayscale images per video with a resolution of 1280 x 1024 pixels. Eight-bit greyscale images are constructed of a 256 colour, or 2^8, greyscale palette that are numbered from 0 to 255 where 0 = black and 255 = white and each number in between is a greyscale gradient between black and white. A grayscale image is more photo sensitive than a colour image and therefore more suitable for filming flies of a species where colour signalling is not present, or at least not of primary interest. The camera could shoot up to 1000 frames per second (fps), but the length of real time video differed between the settings. The constraint to footage length depending on fps setting motivated me to find a compromise between the ability to follow individual particles and film length. Too high fps gave a short film length, and too low fps made it hard to follow objects because of the distance the particles moved between frames. Therefore, I found the best compromise between footage length and the ability to track moving objects at 125 fps (see comparison in Table 1) The footage time was therefore 13.1 seconds. The size of each image is 1.27 MB making each video a little more than 2 GB. The internal memory only allowed to film one segment of footage at a time and the transfer from the camera to the laptop took a several minutes.

fps	Footage length (s)	Comments
75	21.8	Objects hard to follow.
100	16.4	Easy to follow objects, good footage time.
125	13.1	Easy to follow objects, good footage time.
500	3.3	Very easy to follow objects, short footage time.

Table 1 Differences in fps-settings and the possibility to follow individual particles throughout the films.

A total of 13 different movies were filmed at Långsjön and of these only the best three were analysed due to constraints on time for this masters project. The movies were chosen for their clarity (focus point) and because they were filmed at the same position.

I used the image stacks to harness information about the size and velocity of individual flies during swarming by performing image analysis. The first step was to make the images binary, for which I used the software Fiji (Schindelin et al., 2012). This allowed me to differentiate the flies from the background. Since the image had different grades of shading throughout the image, making the image binary by only using a threshold based on a grayscale number was not feasible. Instead, a local threshold approach called the Phansalkar method was applied (Phansalkar et al., 2011) where a threshold was decided depending on the contrast with adjacent pixels. A typical frame from a video from Långsjön can be seen in Figure 2. I specified a centred oval shape with the width of 850 and height of 310 pixels on the binary image where the image analysis would be conducted. The dimensions of the oval shapes were decided for

the purpose of only analysing the part of the images where flies could be detected. Afterwards for each cluster of pixels that differed from the background I extracted its centred Y- and X-coordinates and the size the cluster of pixels occupied. The image-analysis was done for the first image in a video and then every hundredth image in that video, giving 16 analysed images per film. The gap between the images was made for minimising the risk to have multiple data points of the same individual when calculating the number of individuals of each sex and stage of courtship, and for the assistance to find individual flies of interest when tracking was made. Later, when tracking individual flies, I was using all of the images contained in a film. These data were exported from Fiji as a .csv-file and imported into R (R Core Team, 2021). In R the data were reshaped so each data point got its unique ID, what frame it was taken from, its X- and Y-coordinates from that frame and its size, measured in number of pixels that the object occupied.

Figure 2 The same frame from a video at lake Långsjön. Left one being the original image and right one after using the Phansalkar local threshold method to create a binary image.

Assignment of sex and stage of courtship to flies

I expected the flies to be of four different categories based on sex and stage of courtship: swarming females, swarming males, hunting males, and mating pairs. The swarming females would be in the swarm without prey. The swarming males should be carrying a prey item as a nuptial gift and, therefore altering their silhouette to be larger than the swarming females (see Figure 5) (in *H. maura*, there are no known instances in which males without gifts have been found to swarm, L Bussière, pers. comm.). The mating pairs would be two flies and a captured prey together and could be differentiated from males if two sets of wings were detected; they also had a different flying pattern compared to swarming males and females by changing altitude in a more frequent pattern (author´s own observation). The mating pairs would also take turns that were less sharp than the other participants. Hunting males would have a distinctive flying pattern compared to the other categories: while the swarming participants would mostly go in a straight line in the swarming area before turning 180° to fly back in the direction they came from, hunting males would appear to change direction more frequent and in irregular patterns in the search of prey (see Figure 6) (author´s own observation). Out of the four discrete categories, hunting males and swarming females would be the same size but distinguishable by flight pattern when following individuals in a swarm. Hunting males and swarming females would be the smallest group, swarming males the second largest group, and mating pairs the largest group.
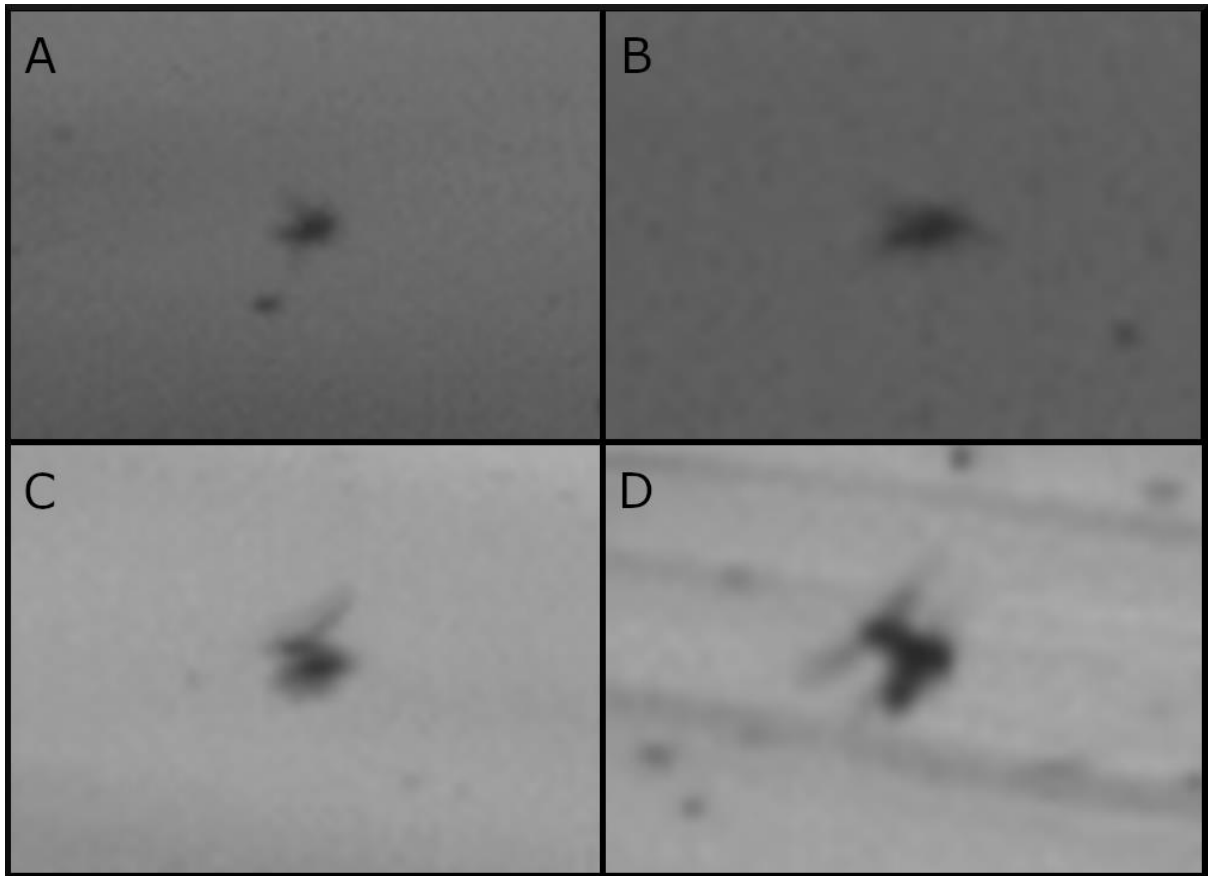
Figure 3 A: Swarming female. B: Hunting male. The differentiation of swarming female and hunting male could not be done by comparing appearances but rather through flight pattern, as was done for these images. C: Swarming male with prey. D: Mating pair, notice the two sets of wings.
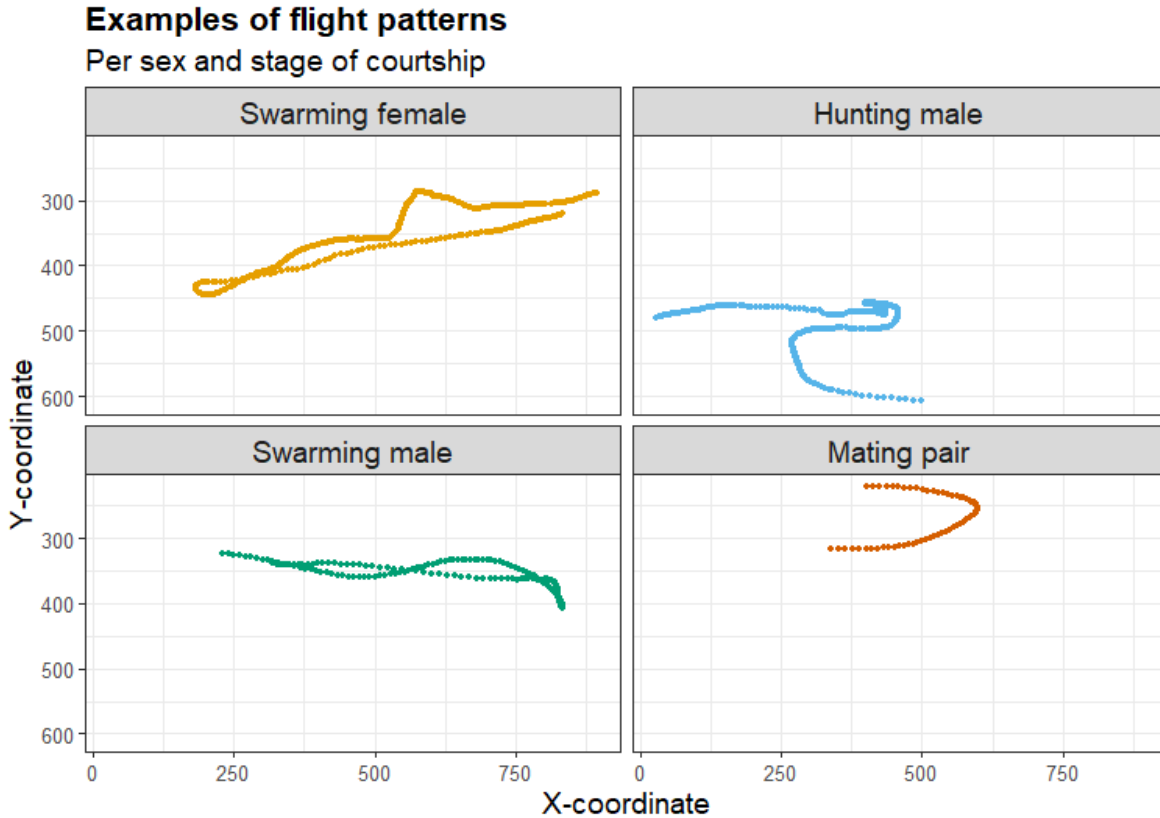
Examples of flight patterns
Per sex and stage of courtship

Figure 4 Examples of flight trajectory of an individual fly from each sex and stage of courtship. Every datapoint in the plots is an instantaneous position of an individual fly during a frame, and each panel illustrates the path for a single representative particle in that class. The swarming individuals will have longer straits that are ended with a sharp turn. Hunting males have a more irregular pattern where the change the direction more often in their hunt for prey. Pairs have a less sharp turn than the other participants. The images are 1280 x 1024 pixels, where the pixel position starts from the left-hand side moving right in the horizontal axis and starts from the upper position moving down in the vertical axis.

Going back to the original images the flies were observed throughout their flying trajectory, not only to notice the different flying patterns but also to make sure that not one fly was counted twice. When a sex and the stage of courtship for an individual fly was confirmed, a new column was created in the dataframe, where the sex and stage of courtship was written down.

After removing particles that were not a *H. maura* fly, I conducted a test for multimodality called excess mass test (Ameijeiras-Alonso et al., 2019) in the R package multimode (Ameijeiras-Alonso et al., 2018) where a test for more than one mode was conducted with a bootstrap of 100 replicates. The multimodality test was made for the purpose of investigating the possibility of categorisation and calculating the counts of the different participants.

Calculation of fly velocities using the Fiji plug-in TrackMate

An estimation of the length of a pixel was made for the purpose of presenting a more comprehensible unit of velocity than pixels per frame. This was done by calculating the pixel length of flies in the middle of the vertical axis and comparing to real life mean size of *H. maura* flies.

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In order to calculate the velocity of the particles and compare between sexes and stages of courtship, I tracked the particles across the sequence of images in a video. I firstly needed to make every image in the video binary using the Phansalkar method (Phansalkar et al., 2011) as explained above, and applying a macro in Fiji that converts all the images in a folder. Thereafter I renamed every image so that the software in the next step would recognise the order. The renaming was done by a self-written script in the Linux console. The images in my video were in .bmp-format and needed to be converted to an .avi-file using the software bmpseq (Miskowski, 2006). I used the Fiji plug in TrackMate (Tinevez et al., 2017) to track the flies. I picked a fly that had previously been assigned to one of the four categories (sex and stage of courtship) and followed its trajectory from its first appearance in the video to its disappearance. Since vertical movement throughout the image (a particle that moves at a constant altitude but towards or away from the camera) in the video would be perceived as much slower than horizontal movement (a particle that moves at a constant altitude and a constant distance from the camera), I would only track flies that flew somewhat horizontally through the images. If the individual had a section of flight that did not deviate more than 45° from the horizontal line, I could proceed to track it with the plug in. I thereafter chose a section of their flight that was without turns to be able to calculate their velocity in a straight line. TrackMate has three options for tracking particles. All three are based on TrackMate recognizing clusters of pixels as particles in a binary image. With both automatic and semi-automatic tracking, the program calculates size differences and distance between particles frame per frame to conduct the tracking. In the automatic tracking the program looks for the most likely tracking outcome for every particle throughout the video selected. When using semi-automatic tracking the user chooses one particle of interest and asks the program to find the most likely track for that particle. The manual tracking still recognises the clusters of pixels as particles, but the user must select two particles in adjacent frames and tell the program to create a link between them. When a flight trajectory has been made in TrackMate the output from the software is three different .csv files whereof two of them were used in the coming steps. One was used to calculate the mean velocity between the categories and the other to compare the velocity in relation to the Y-axis.

To detect and verify the trajectory of the particle that was chosen for tracking I always had the stack of original images open. Each image has information about what frame it comes from in the bottom of the image, which helped when comparing between binary and untampered images. Before starting the tracking, the particle was followed throughout the image to verify that it was possible to be tracked without mistaking it for another particle.

Fully automatic tracking was not possible because of the number of flies in the images and the difference in perceived size they showed in the binary video. For example, the perceived size differed depending on which phase of the wing stroke the fly was in, which interfered with the algorithms used to detect the same particle in subsequent images. Furthermore, flies crossed paths on a regular basis, which interfered with the algorithms ability to track intersecting particles' paths. The semi-automatic tracking was useful in areas with less noise. When noise was too large, because of the number of flies in the given area or because tracked particle was too close to an edge of a shadow, the program either had difficulty finding any particles in the next frame that was possibly the same particle or it would follow the wrong particle when verified using the original image. In these cases, manual tracking was conducted.

Effect on velocity of vertical position

I needed to consider particle position in the Y-axis of the image in relation with its instantaneous velocity to give an adjusted estimate of velocity. The further away an object is from the lens the smaller the object will appear, and therefore will travel fewer pixels per time unit compared to objects closer to the lens when traveling in the same velocity (see Figure 5). If flies fly approximately the same distance from the surface, I expect a negative correlation between the Y-axis and velocity. I assessed whether models that included this change in velocity with vertical position could better explain differences among particle categories than models that ignored the vertical position.
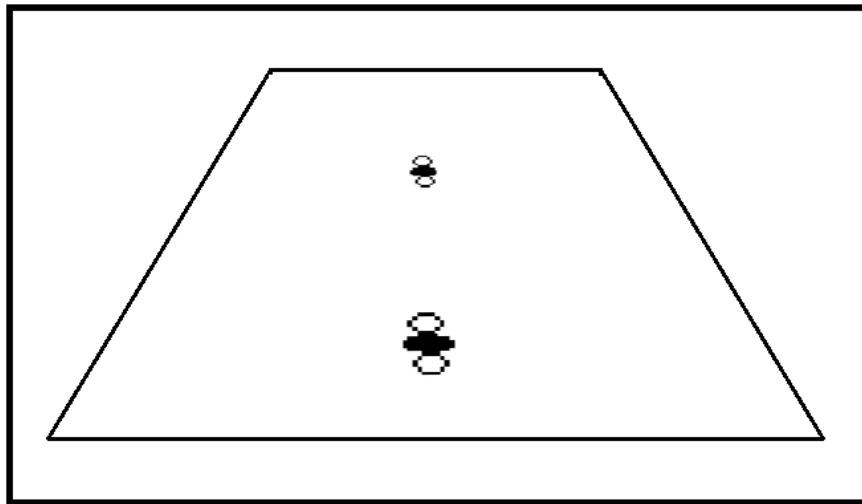
Figure 5 Diagram of image analysed. Both horizontal lines inside the box are the same length in nature but appear to be of different length in the image, causing flies of the same size to appear as different sizes and the velocity to appear to slow down further away from the lens.

To estimate the partial effect of vertical position, I fitted linear mixed models where each individual fly´s Y-coordinate was a predictor of its instantaneous velocity for each of their recorded frames and was used to assess speed across different sexes and stages of courtship. I included individual identity as a random effect to account for repeated measures on particles and allowed the effect of vertical position to depend on the particle´s identity, in recognition of the possibility that the changes in vertical position by distance might differ depending on the individual. The decision was made to add individuals' vertical location as a crossed random effect in the model instead of assuming that the effect on velocity on vertical position is the same for each fly after observing substantial variation in the vertical movement of the flies depending on individual.

I compared nested mixed models with and without each term of interest to determine whether fixed effects were important using parametric bootstraps implemented in the R package pbkrtest (Halekoh & Højsgaard, 2014).

Results

Initial test using mobile phone at Sisjön

The initial filming at Sisjön was not suitable for any further video analysis. The low resolution and the difficulty to find the correct focus point made it very hard, if not impossible, to differentiate between the sexes and stages of courtship (see Figure 6). For this reason I made arrangements for a short-term hire of a professional high-speed camera.

Figure 6 A frame from the first set of videos shot at Sisjön. The dimension of the tarp is 3x5 m. Later we used two tarps of the same size next to each other. The white dots seen against the blue tarp are Rhamphomyia crassirostris flies.

Multimodality test for ratio of the different sexes and stage of courtship

The initial multimodality test was made using one movie with 354 individual particles as seen in Figure 7. All particles that were smaller than six pixels in area were removed prior to the test since a majority of the small particles were noise that was created at the edges between the dark and illuminated areas seen in Figure 2. The data were stripped of non-*H. maura* flies before creating the graph but not the reflections of dance flies that are seen on the water surface. Reflections on the water surface could easily be detected if looking through the full footage by following individual flies and noticing identical particles mirroring their movement beneath them. On a still image, however, it was impossible to discern reflections for flies unless the reflection was distorted by a small wave. There was no support for multimodality as can be seen in Figure 7. Even though different sexes and stages of courtship represent different sizes, they could not easily be discerned into several size classes. Instead, the distribution of particles revealed a single mode at the smallest particle size that gradually tapered in frequency with increasing size. There was no evidence of more than one mode in the excess mass test (Ameijeiras-Alonso et al., 2019) (Excess mass = 0.03, p=0.21) .

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Observed particles
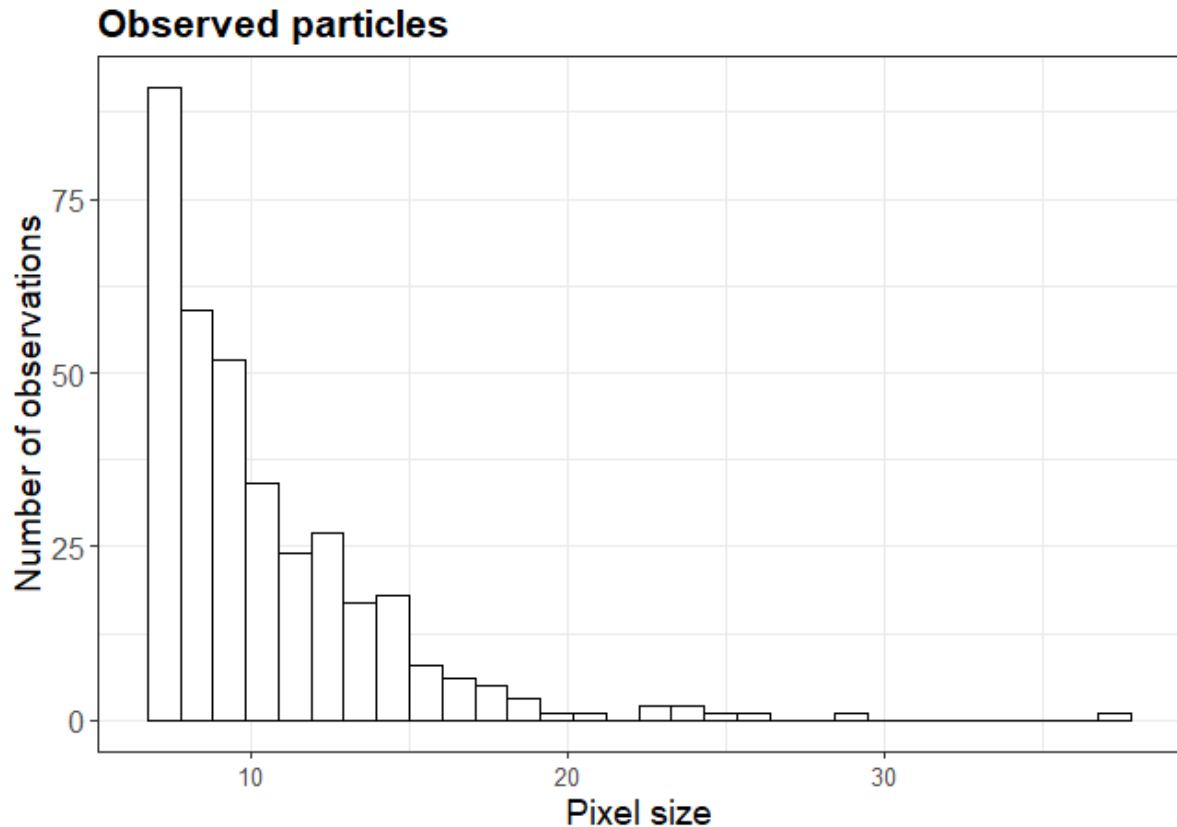
Figure 7 Histogram with number of individuals on Y-axis and size in pixels on X-axis. Particles bellow the area size of six pixels were not included. The outliers in the larger spectrum are flies that flew close to the camera.

Velocity by sex and stage of courtship

I estimated the actual length of a pixel since length markers were absent in the field. The estimation was done for the purpose of having a unit of velocity that is more easily comprehensible than pixels per frame. Flies close to the surface level in the vertical centre of the image varied between 3-5 pixels. A *H. maura* fly body is approximately 0.8 cm in length and therefore the conclusion was made that one pixel is approximately 2 mm in length.

Objects further away from the camera will appear smaller and slower in the filmed material than they actually are in the field. To correct for this, I calculated the instantaneous velocity based on vertical position using linear mixed models (see Table 2).

Velocities in thirteen swarming females, ten swarming males, five hunting males, and two mating pairs were analysed. The linear mixed model that was used fits an individual fly´s instantaneous velocity as a function of group and vertical location, including a random effect to account for repeated measurements of individuals, and a crossed random effect of vertical position that allows its importance to vary by fly. This crossed random effect was added because of the variety flies show in their movement in vertical position as illustrated in Figure 8. The datapoints are picked frame by frame while tracking the flies and therefore there will be numerous datapoints per fly present in the plots seen in Figure 9.

| Variance components		Variance	Std deviation	Corr	PBtest p
Individual	Intercept	1.4446	1.2019		
	Vertical position	0.9151	0.9566	-0.70	0.001
Residual		0.2578	0.5077		
Fixed effects		**Estimated velocity**	**Std error**	**t-value**	**PBtest p**
Intercept		1.8630	0.2957	6.299	
Vertical position		0.4318	0.1857	2.326	
Sex and stage of courtship (Hunting male)		-0.5099	0.4871	-1.047	
Sex and stage of courtship (Swarming Male)		0.4511	0.3829	1.178	0.062
Sex and stage of courtship (Pair)		1.8071	0.8113	2.227	

Table 2 Linear mixed model with each individual's instantaneous velocity in meters per second and individual change in perceived velocity depending on vertical position as random effects. Vertical position is the slope that is shared for each sex and stage of courtship. The estimates show the velocity in the centre of the image for the first group and each other sex and stage of courtships estimate is the additive velocity for the same position. The reference group for sex and stage of courtship is swarming females. The PBtest is the p-value for a parametic bootstrap test with and without the parameter of interest with 1000 replicates, note that it applies collectively to the predictor for the sex and stage of courtship as indicated by the shaded cell in the table.
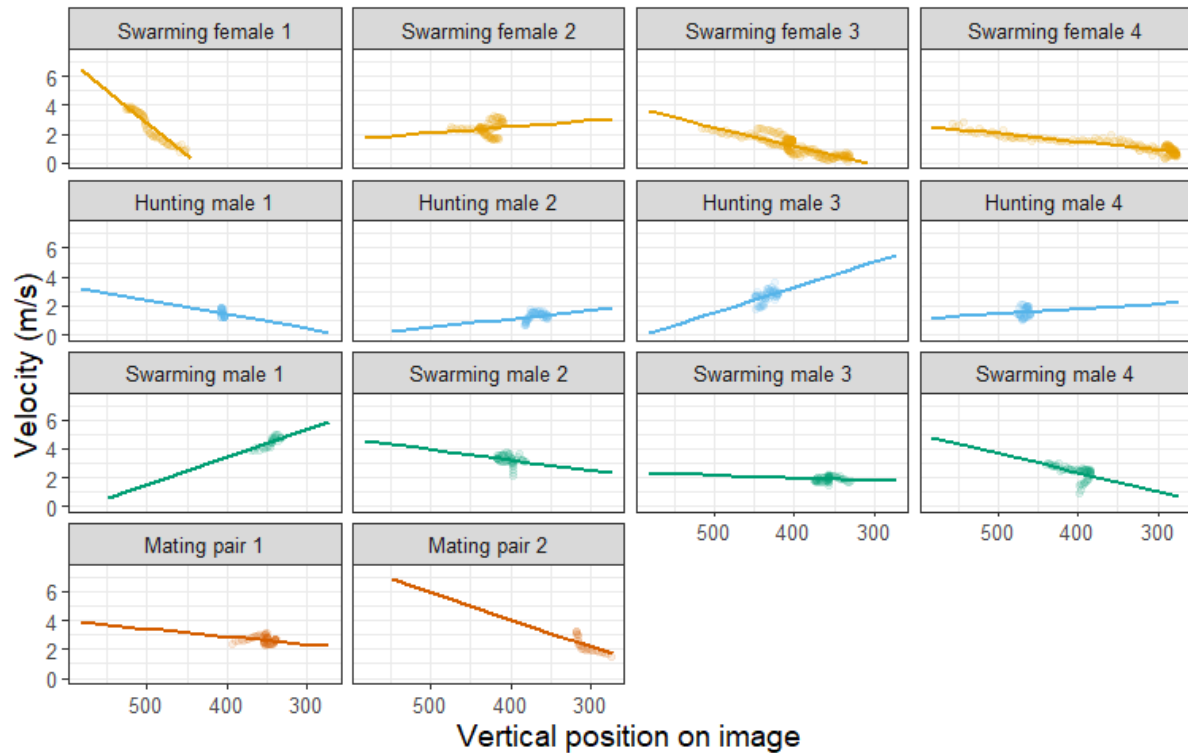
Figure 8 Individual variance in velocity change depending on vertical position. The Y-axis shows velocity in meters per second, the X-axis shows the Y-axis location in the filmed image. This plot illustrates four representative individuals of each sex and stage of courtship except mating pairs, where only two particles were found in the videos of interest. Each datapoint in each graph show the instantaneous velocity and vertical position for that individual. The trendline gives the expected velocity change with change in vertical position for each individual according to the linear mixed model. The images are 1280 x 1024 pixels, where the pixel position starts from the left-hand side moving right in the horizontal axis and starts from the upper position moving down in the vertical axis.

There was a marginally non-significant difference (p = 0.062) between the sexes and stages of courtship in the model according to a parametic bootstrap test. In light of the limited power available for our analysis due to a low sample size, this amounts to weak evidence for differences in velocity, as disussed below. There was however a significant difference (p = 0.001) when the same test was made between a model with and without individual change in preceved velocity depending on vertical location as a crossed random effect.

The linear mixed model show that pairs were the fastest (mean 3.7 m/s, SE = 0.8) in the central vertical position of the image, swarming males, the second fastest (mean 2.3 m/s, SE = 0.4), swarming females third (mean 1.9 m/s, SE = 0.3), and hunting males the slowest (mean 1.4 m/s, SE = 0.5). Visualisation of the raw data and the fitted effects can be seen in Figure 10. The small sample size of hunting males and mating pairs means that the fitted effect should be interpreted cautiously, especially in the light of the poor fit and elevated altitude of the mating pairs.
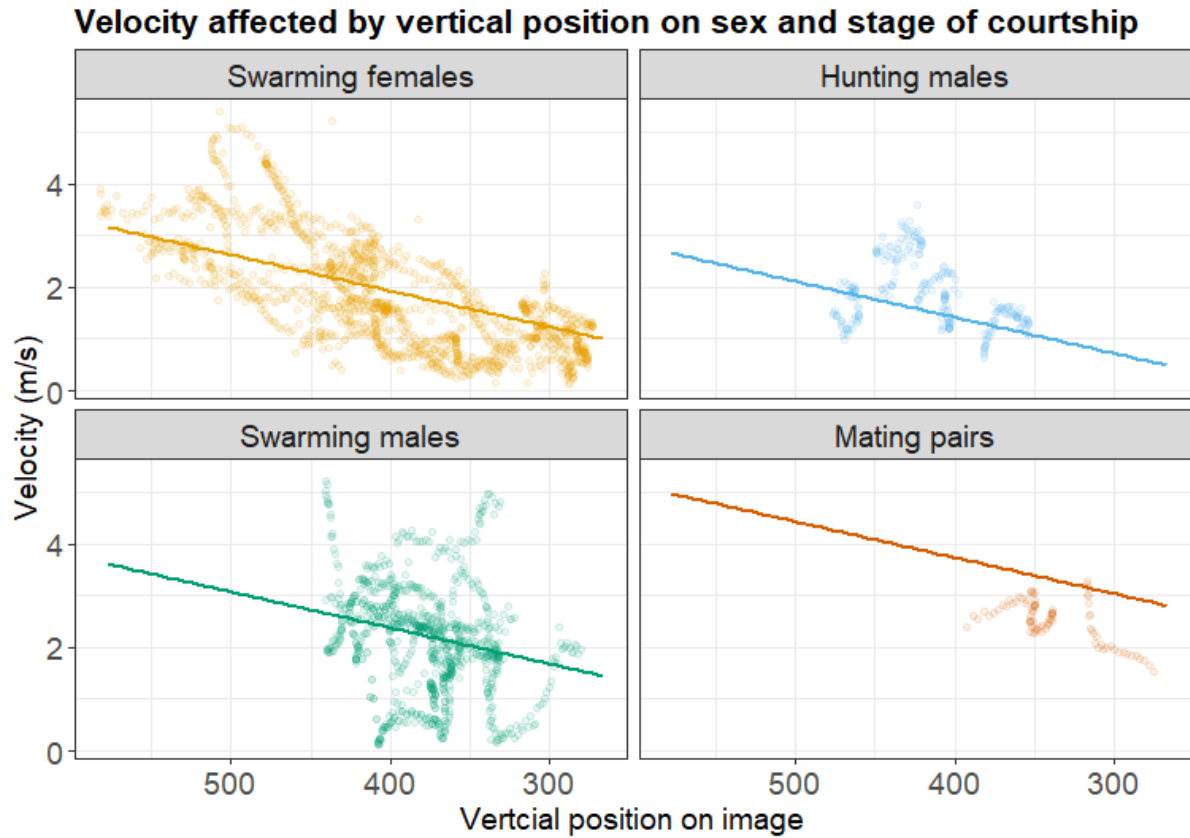
Figure 9 Linear mixed model with each individual's instantaneous velocity in meters per second and individual change in perceived velocity depending on vertical position as random effects. There are 13 swarming females, ten swarming males, five hunting males and two mating pairs. Each graph Y-axis show the velocity in meters per second and X-axis show the location on vertical position. Each replicate in the plots is an individual fly's instantaneous vertical position and velocity for that position and since the flies were tracked throughout several frames they will have as many datapoints in the plots. The images are 1280 x 1024 pixels, where the pixel position starts from the left-hand side moving right in the horizontal axis and starts from the upper position moving down in the vertical axis.
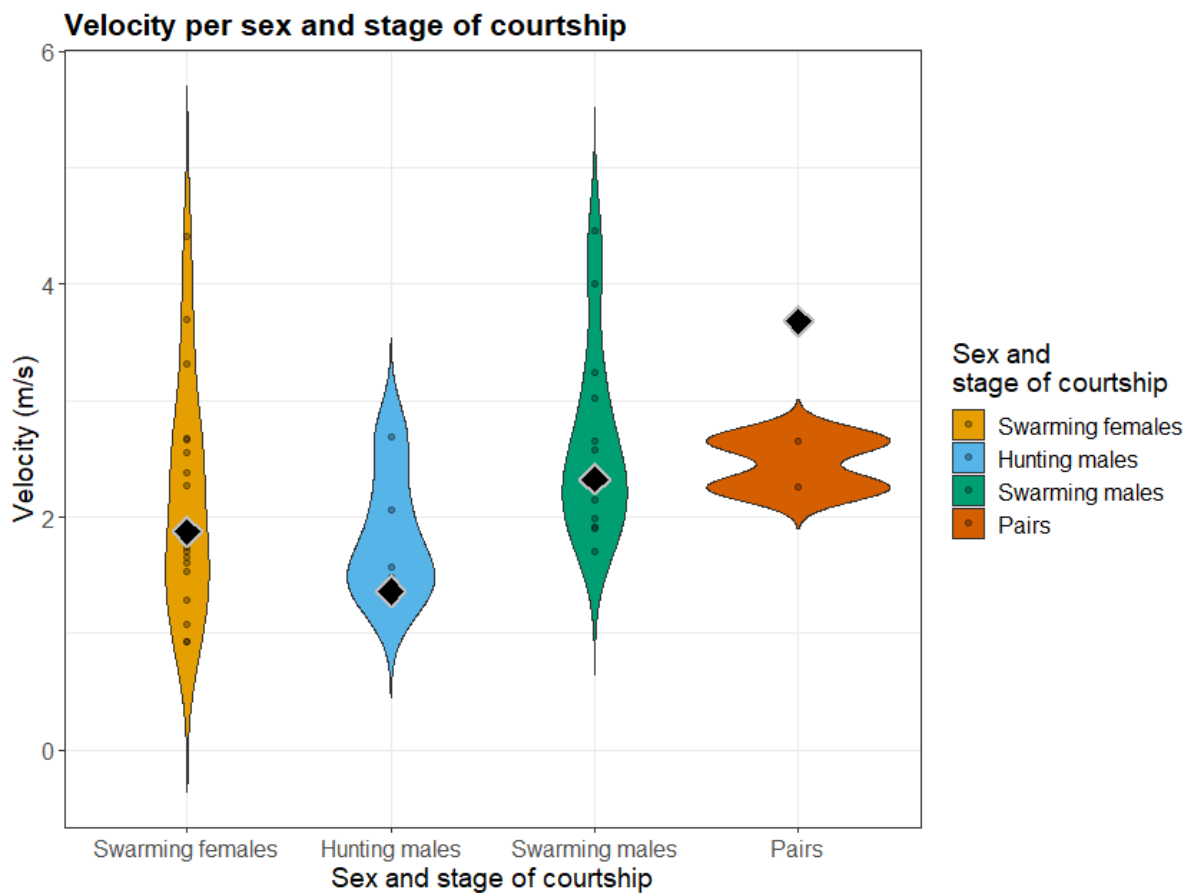
Figure 10 The violin plot shows the average velocity of the raw data. The points inside each plot is individual mean velocities. The diamond in each plot is the fitted estimated velocity for said sex and stage of courtship the central vertical position of the image when controlled for vertical position. The reason the fitted value for pairs is far away from the two observations relates to the high positions of pairs in vertical space, and the small sample size.

Discussion

Possible type of selection acting on intersexual differences

My results show marginally non-significant differences in velocities between the sexes and stages of courtship, with mating pairs being the fastest, followed by swarming males, swarming females, and hunting males as the slowest group. No apparent variation differences could be found between the categories. Although the results were statistically non-significant the trends are suggestive and justify further study and discussion about the four different hypotheses presented in the aims.

a) Predation avoidance or hunting success. If the variation in velocity between the sexes and stages of courtship would be caused by predation avoidance or hunting success, I expected hunting males to be the fastest group since they are the only ones that hunt and might experience increased predation risk during hunting (Cumming, 1994). There would also be a larger variation in velocities within hunting males since the need for acceleration would not be constant. This hypothesis can be ruled out due to hunting males being the slowest group.

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b) Load lifting constraint. With the load lifting constraint hypothesis, I expected mating pairs to be the slowest group and swarming males being the second slowest due to constraints from the carried weight of the prey item (Marden, 1989). Swarming males being faster than both swarming females and hunting males indicates that the added weight of the caught prey item did not substantially slow the swarming males down and the maximum load lift is not achieved. This result is consistent with Murray et al.´s (2019) finding of load lifting constraint not affecting male reproductive success in a different dance fly species.

c) Intrasexual competition through scramble competition for females. In male scramble competition for females, I would expect swarming males being the fastest group. This is consistent with my findings. However, as I will be discussing in the next paragraph, there is not a larger variation in velocities in the swarming male category than the others as I would expect if this hypothesis was to be true. In scramble competition I would expect males to swarm in a pace that was similar to the other participants until they noticed a recipient female and would then accelerate to win the race to approach her before another male.

d) Intrasexual competition through mate choice. This hypothesis has the same expected velocity ranking as hypothesis c but one way to detect the difference between the two main hypotheses about intrasexual competition might be by studying the variation in velocities within groups. We know that swarming males are faster than swarming females. Therefore, there is possibly something that acts on stronger selection for speed in males than in females. If the females get approached by the males inside of swarms, there is no obvious reason for individual females to differ among themselves in velocities when inside the swarm. The same could be said for swarming males if female choice is the reason for intrasexual competition. As long as males occupy swarms, they should be doing their best to attract mates, and if speed is a way of showcasing fitness as Sullivan (1981) suggests, there is no good reason for males to vary substantially in speed. If, however the difference is born from scramble competition, we would except swarming males to accelerate upon detecting a female as males race to be the first to pair with her. These races are expected in scramble systems characterise by male-biased swarms, and often occur in species with exaggerated male sensory organs for detecting females like the dichoptic eyes of dance flies (Downes, 1969; Wiberg et al., 2021). However, my data do not provide any evidence for substantial sex differences in within-group variation in flight speeds: in contrast, there is at least as much evidence for changes in velocity among females within Figure 10 as for changes among males. The extent to which this is partly caused by differences in depth is not clear, but the mixed model approach was designed at least in part to minimise the effect of distance on variation in speed. More research is needed to comprehensively rule out scrambles, but my preliminary findings at least do not strengthen the likelihood that they explain important variation in behaviour within swarms of *H. maura*.

The argument for male choice being active could be made as well. Assume that males want to mate with the most fecund females. The most fecund females should be full of eggs and therefore the ones that are the plumpest and consequently resulting in fecund females being slower than the other groups. With the results showing no obvious constraint on load lifting in males it is not likely that females would exhibit one either. An honest signal like this would also be easy to abuse since it is a lot easier to achieve slower velocities as an individual than it is to achieve faster ones and we would except larger differences between the sexes.

One possibility that is consistent with my findings is that there is female mate choice acting on velocities inside swarms. With swarming males being the fastest without displaying different in-group variation in speeds with other groups the results can be interpreted as

female preference for faster males. This is interesting in a species like *H. maura* where males have a lot to gain by putting in effort to choose the correct mate for his reproductive services, when it is widely believed that dance fly females mate with multiple mates in the effort to gather multiple nuptial gifts (Arnqvist & Nilsson, 2000). Remember that the gift males present to females is the only way adult female dance flies gets their protein. If female mate choice is acting on male velocity is the correct assessment it does not mean there are not male mate choice active on other traits such as size. As discussed in the introduction, there are different traits that males could prefer in dance flies, as supported by the presence of female ornaments in many dance fly species (Funk & Tallamy, 2000; LeBas et al., 2003; Murray et al., 2018; Svensson & Petersson, 1988; Svensson et al., 1989). Female ornaments are absent in *H. maura* however, which means that I cannot easily extend my findings to other species.

The fact that my model predicts that mating pairs as the fastest of the participants is most likely a reflection of their high position above the water surface together with a low sample size, rather than a true difference in velocity for mating pairs. Mating pairs were not easy to find in the footage and when they were found they often only occupied the film for a short period of time. The reason of high vertical position in mating pair would be true even with a larger sample size, as the model assumes that all participants are at the same altitude above the lake surface, and which will skew the predictions.

Interestingly, hunting males were the slowest group. One reason for this could be that there is not a need to accelerate to high speeds when hunting for prey if the preyed species are slower or more stationary than the hunting males. The result can again be because of low sample size but another reason can be the way hunting males were detected. They were detected by their flight pattern and their turns were more frequent than the swarming participants. Making their straight lines shorter and the possibility is that they did not accelerate to full speed. Even so, it seems plausible that high speeds are not necessary for hunting males.

The possibilities of using high-speed footage in swarm studies

Even though filming small moving objects such as dance flies can be troublesome it still is possible. Both species I filmed during the summer had its own set of constraints. *Rhamphomyia crassirostris* was filmed in front of a tarp, and we had to be careful when installing the tarps against the trees since small disturbances would make the swarm change location. When it came to *Hilara maura* the problem was different since they occupy the space just above the water surface. The depth issue (distance between flies and the lens) became evident when the test for multimodality was made since a conclusive modality pattern was not found and yet is likely to exist in the real size distribution of particles.

Future filming of *H. maura* swarms could benefit from a multiple-camera setup. This is expensive since the retail price for the camera that was used is over 100,000 SEK and financial constraints are always present. Multiple cameras filming simultaneously would give the opportunity to recreate the swarm in 3D. A birds-eye view would be another interesting method that could be tried where the camera is situated directly above the swarm. This could be difficult to succeed without disturbing the flies but not necessarily impossible. You would lose the level of altitude change but to have both vertical and horizontal axis clearly visible would still be more preferred. *R. crassirostris* could be filmed with better equipment since the solution with tarps situated between trees worked well when the tarp didn't disturb the flies. There would still be the depth issue in a one-camera approach.

Future work

In broader sense there is still much unknown about dance flies. We do not know why some species develop female ornaments and some do not, and it would be interesting to test velocities between the sexes and stages of courtship in other species of dance flies, in an array of species that show and do not show female ornamentation as well as comparing their operational sex ratios to see if there are any patterns that could be detected. There is also work to be done in investigating how the transfer of nuptial gifts happen. I was not able to catch the transfer in my footage but if the possibility for longer footage periods emerged (e.g., by high-speed data transfer from a camera directly to a storage device) this could be done.

Conclusions

Males in *H. maura* appear to be moderately (albeit non-significantly) faster than females, but the variation in speeds is similar between the sexes. These findings are consistent with the idea of having intersexual differences in performance traits for the purpose of showcasing performance for the opposite sex. It is also consistent with previous knowledge of the OSR being slightly male biased in *H. maura* (Murray et al., 2020), and the more abundant sex (males) competing for the other. Much more work is needed to confirm and clarify how flight performance and behaviour relate to mating systems in this group. My thesis has illustrated that despite the difficulties inherent with studying fast-moving swarms, there are opportunities to use technology and data science to address some important questions about the diversity in this fascinating group.

Acknowledgements

References:

Alcock, J. (1980). Natural selection and the mating systems of solitary bees. *American Scientist, 68*(2), 146-153.

Ameijeiras-Alonso, J., Crujeiras, R. M., & Rodríguez-Casal, A. (2018). Multimode: An R package for mode assessment. *arXiv preprint arXiv:1803.00472*.

Ameijeiras-Alonso, J., Crujeiras, R. M., & Rodríguez-Casal, A. (2019). Mode testing, critical bandwidth and excess mass. *Test, 28*(3), 900-919.

Andersson, M. (1982). Female choice selects for extreme tail length in a widowbird. *Nature, 299*(5886), 818-820. doi:https://doi.org/10.1038/299818a0

Arnqvist, G., & Nilsson, T. (2000). The evolution of polyandry: multiple mating and female fitness in insects. *Animal behaviour, 60*(2), 145-164. doi:https://doi.org/10.1006/anbe.2000.1446

Bonduriansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews, 76*(3), 305-339.

Chvala, M. (1996). A taxonomic revision of the Hilara maura-group (Diptera: Empididae) in Europe. *Systematic Entomology, 21*(4), 265-294. doi:https://doi.org/10.1111/j.1365-3113.1996.tb00601.x

Chvála, M. (1976). Swarming, mating and feeding habits in Empididae (Diptera) and their significance in evolution of the family. *Acta entomologica Bohemoslovaca, 73*, 353-363.

Clutton-Brock, T. (1982). The functions of antlers. *Behaviour, 79*(2-4), 108-124.

Cumming, J. M. (1994). Sexual selection and the evolution of dance fly mating systems (Diptera: Empididae; Empidinae). *The Canadian Entomologist, 126*(3), 907-920. doi:http://dx.doi.org/10.4039/Ent126907-3

Downes, J. (1969). The swarming and mating flight of Diptera. *Annual review of entomology, 14*(1), 271-298.

Downes, J. A. (1970). The feeding and mating behaviour of the specialized Empidinae (Diptera); observations on four species of rhamphomyia in the high arctic and a general discussion. *The Canadian Entomologist, 102*(7), 769-791. doi:10.4039/Ent102769-7

Edward, D. A. (2014). The description of mate choice. *Behavioral Ecology, 26*(2), 301-310. doi:10.1093/beheco/aru142

Eltringham, H. (1928). On the Production of Silk by Species of the Genus Hilara Meig. (Diptera). *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 102*(718), 327-334.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*(4300), 215-223.

Fitzpatrick, S., Berglund, A., & Rosenqvist, G. (1995). Ornaments or offspring: costs to reproductive success restrict sexual selection processes. *Biological Journal of the Linnean Society, 55*(3), 251-260. doi:https://doi.org/10.1016/S0024-4066(95)99999-X

Funk, D. H., & Tallamy, D. W. (2000). Courtship role reversal and deceptive signals in the long-tailed dance fly, Rhamphomyia longicauda. *Animal behaviour, 59*(2), 411-421.

Gwynne, D., & Bussière, L. (2002). Female mating swarms increase predation risk in a'role-reversed'dance fly (Diptera: Empididae: Rhamphomyia longicauda Loew). *Behaviour, 139*(11-12), 1425-1430.

Gwynne, D. T., Bussière, L. F., & Ivy, T. M. (2007). Female ornaments hinder escape from spider webs in a role-reversed swarming dance fly. *Animal behaviour, 73*(6), 1077-1082.

Halekoh, U., & Højsgaard, S. (2014). A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models the R package pbkrtest. *Journal of Statistical Software, 59*(1), 1-32.

Hamm, A. H. (1928). On the epigamic behaviour of Hilara maura, Fab., and two allied species. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 102*(718), 334-338.

Herridge, E., Murray, R. L., Gwynne, D., & Bussiere, L. (2016). Mating and Parental Sex Roles, Diversity in. *Encyclopedia of Evolutionary Biology, 2*. doi:10.1016/B978-0-12-800049-6.00154-2

Hockham, L. R., & Ritchie, M. G. (2000). Female secondary sexual characteristics: appearances might be deceptive. *Trends in Ecology & Evolution, 15*(11), 436-438.

Hunter, F. D., & Bussière, L. F. (2019). Comparative evidence supports a role for reproductive allocation in the evolution of female ornament diversity. *Ecological Entomology, 44*(3), 324-332.

Kessel, E. L. (1955). The mating activities of balloon flies. *Systematic zoology, 4*(3), 97-104.

Kokko, H., Klug, H., & Jennions, M. D. (2012). Unifying cornerstones of sexual selection: operational sex ratio, Bateman gradient and the scope for competitive investment. *Ecology Letters, 15*(11), 1340-1351.

Kvarnemo, C., & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution, 11*(10), 404-408.

Lailvaux, S. P., Alexander, G. J., & Whiting, M. J. (2003). Sex-based differences and similarities in locomotor performance, thermal preferences, and escape behaviour in the lizard Platysaurus intermedius wilhelmi. *Physiological and Biochemical Zoology, 76*(4), 511-521.

LeBas, N. R., & Hockham, L. R. (2005). An invasion of cheats: the evolution of worthless nuptial gifts. *Current Biology, 15*(1), 64-67.

LeBas, N. R., Hockham, L. R., & Ritchie, M. G. (2003). Nonlinear and correlational sexual selection on 'honest'female ornamentation. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1529), 2159-2165.

Marden, J. H. (1989). Effects of load-lifting constraints on the mating system of a dance fly. *Ecology, 70*(2), 496-502.

Miskowski, R. (2006). BMP to AVI Sequencer. Retrieved from https://sourceforge.net/projects/bmpseq/

Murray, R. L., Gwynne, D. T., & Bussière, L. F. (2019). The role of functional constraints in nonrandom mating patterns for a dance fly with female ornaments. *Journal of Evolutionary Biology, 32*(9), 984-993.

Murray, R. L., Herridge, E. J., Ness, R. W., Wiberg, R. A. W., & Bussière, L. F. (2020). Competition for access to mates predicts female-specific ornamentation and male investment in relative testis size. *Evolution, 74*(8), 1741-1754.

Murray, R. L., Wheeler, J., Gwynne, D. T., & Bussière, L. F. (2018). Sexual selection on multiple female ornaments in dance flies. *Proceedings of the Royal Society B: Biological Sciences, 285*(1887), 20181525.

Phansalkar, N., More, S., Sabale, A., & Joshi, M. (2011). *Adaptive local thresholding for detection of nuclei in diversity stained cytology images.* Paper presented at the 2011 International conference on communications and signal processing.

Preston-Mafham, K. G. (1999). Courtship and mating in Empis (Xanthempis) trigramma Meig., E. tessellata F. and E.(Polyblepharis) opaca F.(Diptera: Empididae) and the possible implications of 'cheating'behaviour. *Journal of Zoology, 247*(2), 239-246.

R Core Team. (2021). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Sadowski, J. A., Moore, A. J., & Brodie III, E. D. (1999). The evolution of empty nuptial gifts in a dance fly, Empis snoddyi (Diptera: Empididae): bigger isn't always better. *Behavioral Ecology and Sociobiology, 45*(3), 161-166.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods, 9*(7), 676-682. doi:10.1038/nmeth.2019

Shuker, D. M., & Kvarnemo, C. (2021). The definition of sexual selection. *Behavioral Ecology*.

Sullivan, R. T. (1981). Insect swarming and mating. *The Florida Entomologist, 64*(1), 44-65.

Svensson, B. G. (1997). Swarming behavior, sexual dimorphism, and female reproductive status in the sex role-reversed dance fly species Rhamphomyia marginata. *Journal of Insect Behavior, 10*(6), 783-804.

Svensson, B. G., & Petersson, E. (1987). Sex-role reversed courtship behaviour, sexual dimorphism and nuptial gifts in the dance fly, Empis borealis (L.). *Annales Zoologici Fennici, 24*(4), 323-334.

Svensson, B. G., & Petersson, E. (1988). Non-random Mating in the Dance Fly Empis borealis: The Importance of Male Choice. *Ethology, 79*(4), 307-316. doi:https://doi.org/10.1111/j.1439-0310.1988.tb00719.x

Svensson, B. G., & Petersson, E. (1995). Diurnal and seasonal variations in swarming and mating behaviour of the dance fly Empis borealis (Diptera:Empididae). *Annales Zoologici Fennici, 32*(4), 403-409.

Svensson, B. G., Petersson, E., & Forsgren, E. (1989). Why do males of the dance flyEmpis borealis refuse to mate? The importance of female age and size. *Journal of Insect Behavior, 2*(3), 387-395.

Thornhill, R. (1979). Adaptive female-mimicking behavior in a scorpionfly. *Science, 205*(4404), 412-414.

Tinevez, J.-Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., . . . Eliceiri, K. W. (2017). TrackMate: An open and extensible platform for single-particle tracking. *Methods, 115*, 80-90. doi:https://doi.org/10.1016/j.ymeth.2016.09.016

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In B. Campbell (Ed.), *Sexual Selection and the Descent of Man* (pp. 136-179): Aldine-Atherton.

Wiberg, R. A. W., Murray, R. L., Herridge, E., Gwynne, D. T., & Bussière, L. F. (2021). *Sexually antagonistic co-evolution can explain female display signals and male sensory adaptations*. Unpublished manuscript. Biological and Environmental Sciences. University of Stirling.

Young, J. H., & Merritt, D. J. (2003). The ultrastructure and function of the silk-producing basitarsus in the Hilarini (Diptera: Empididae). *Arthropod structure & development, 32*(2), 157-165. doi:10.1016/S1467-8039(03)00006-9

Appendix

Appendix 1 Popular science summary

The love dance of the dance fly

The mating behaviour of the dance flies has been notoriously hard to study, mainly because of the speed they move in their swarms, and with the help of a high-speed camera we might take a step closer to understanding these peculiar animals.

Evolution has found incredible ways of differentiating between males and females in many species in the animal kingdom. This diversity can sometimes be hard to explain. One notable example is the vide variety of extravagant colours shown in male birds, where their female counterparts usually have a more modest appearance. These differences are often attributed to something we call sexual selection. Sexual selection comes down to that some individuals in a species will have a higher chance to reproduce than others because of characteristics that are used to either attract members of the opposite sex or to out compete members of the same sex. Often sexual selection is the strongest in males because females need to form eggs that are more resource costly than sperm and, in many species, have a larger parental investment with pregnancy and/or parental care. This means females have a larger incentive to be choosy when it comes to choosing a partner than males.

A group of animals that are less known to the public but have big differences between the sexes are the dance flies. The dance flies biggest claim to fame is their elaborate courtship behaviour. Dance flies gather in swarms in the summer for the purpose of mating. In these swarms the male will present a nuptial gift to the female, often in the form of prey, sometimes wrapped in silk. If the female accepts the gift, she will eat it and copulation will take place.

This behaviour is true for most of the approximately 1,450 species of dance flies worldwide. But in around a quarter of these species the females have some sort of secondary characteristic that differentiates them from the males. These characteristics include feathery legs, enlarged or darkened wings, and inflatable stomachs. Most (if not all) of them appear designed to give males the impression that they are larger than they actually are, perhaps to fool the males to think they are filled with eggs and therefore appropriate candidates to offer their gift and being the parent of their future offspring. It makes you wonder why some species of dance flies have female ornaments, but others do not, and if there are any other differences between the sexes that are hidden from the naked eye.

The large gaps in dance fly knowledge are partly due to their behaviour is needed to be studied in the field since they do not behave as expected in a lab environment. To overcome this problem, I have used high-speed camera equipment to film a swarm of a species of dance flies that do not have apparent sexual characteristic differences. I then analysed the footage with help of computer software to find out that males that swarm are both faster than females that swarm and males that hunt. Although these differences are compelling, and my findings preliminary, one possibility that is consistent with these findings is that fast males have an advantage in mating contest, perhaps due to female preference to speed.

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