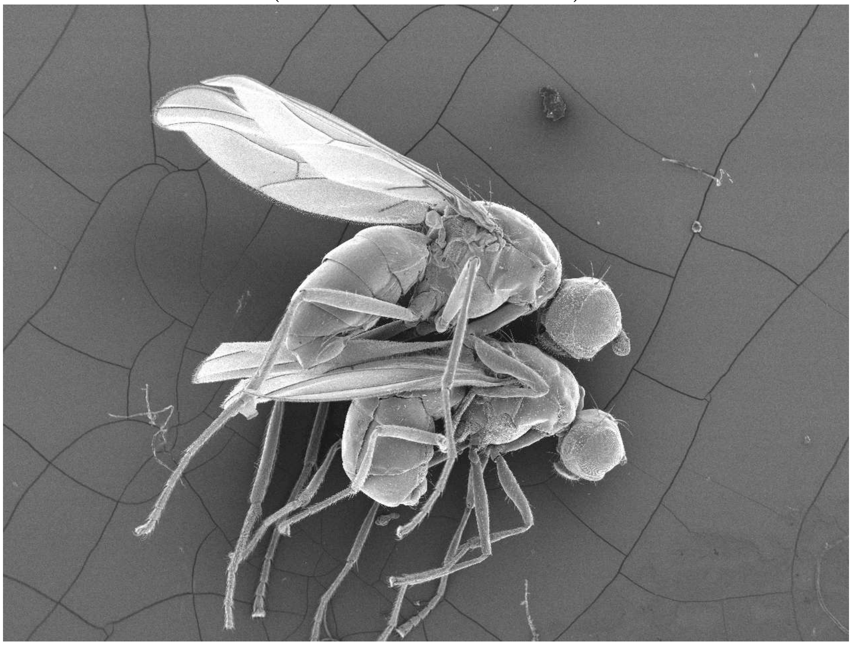
# Fly Flirts

A study of precopulatory behaviour in genus *Themira*

(Diptera: Sepsidae)



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# 1 Introduction

*"Nothing in biology makes sense except in the light of evolution"*

– Theodosius Dobzhansky (1900-1975)

Flirting might seem like a strange subject to investigate scientifically, particularly on a species as unromantic as the dung fly. In order to put our study into context, we must first examine the principles of natural selection which lead to flirting in the first place.

The process of evolution relies largely on sexual recognition, and the idea of speciation relies on specialization of sexual preference. The idea that in the differentiation of species not evolved from an allopatric speciation evolution, a certain event must have occurred that divided the families and caused inter-special breeding to discontinue. In this case, speciation is thus the evolution of mate recognition.

In the process of mate recognition, communication between the sexes will therefore play an integral part in the evolutionary process. Sexual selection determines the genetic integrity of the species and its eventual differential into species. Thus the capability of a species to select which member of the opposite sex that it would like to mate with is an important step in maintaining the presence of healthy progeny for many generations to come.

Once speciation has occurred, communication will thus ensure the capability of distinct species identifying each from one another. In the initiation of sexual interaction, it is generally acknowledge that males of a large percentage of species will actively seek out the female in mating. A large expense of energy is therefore expended by the male in the communicative need for mating, and for identifying the correct species.

In humans, it is relative easy to identify one sex from another. Visual and aural clues play a large part in the communicative process between humans in sexual recognition and eventual mating; not counting psychological and sociological aspects of the situation. In humans, mating is really complex, (not to mention heartrending, mind-numbing, soul-jerking, death defying) process.

In the world of insects and in particular flies, the process of mating is thus simplified to the ideas of mate recognition and the eventual courtship process.

This study would thus like to investigate the mating recognition in the dung flies Themira minor and Themira lucida. The two flies are commonly found in the northern hemisphere and they would often coexist together around the same habitat.2 Both of the flies they are morphologically very similar thus it is difficult to differentiate them simply by visual cues. A means of mate recognition must be used by the two flies to circumvent accidental mating between the two species.

The focus will be on the mating behaviour of the two flies, as it was observed that a specific precopulatory mating process occurs between the male and female flies before copulation occurs we would like to elucidate what is the process specifically. This is because communication occurs between the two sexes during this time which would lead to the males and females selecting the best mate in which it would copulate with.

Further research needs to be done to investigate the interaction when a *T. lucida* meets a *T. minor* .

# 2 Materials and Methods

## 2.1 Species used

***Themira minor*** (Haliday, 1833)

Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: Sepsidae, Subfamily: Sepsinae, Genus: Themira, Species: *Themira minor*

The main diagnostic features of the species are, in the adult, the absence of postpronotal setae, pruinose katepisternum, ornamentation of the male forelegs and the presence of a short expanded posterior seta on tarsomere 4 of the male mid leg. The presence of a short but expanded posterior seta on tarsomere 4 of the male mid leg is the main distinguishing feature between *T. minor* and *T. lucida* [1].

In the adult fly, the head, antenna, thorax, scutellum, legs and abdomen are entirely black. Only the fore coxa and most of trochanters are yellow brown. The wings are clear calypter white, with a creamy margin, fringe hairs and haltere.

These flies are commonly distributed in northern areas of Europe (such as Denmark and Norway), and are widespread in the Palaeartic areas [2]. It is usually found in damp habitats and meadows, and usually breed in dungs, ie. Cow dung, pig dung etc [3][4]. In urban areas they are commonly seen in cattle feeding pens [5] and sewage treatment plants [6].

The males show a territorial behaviour and shows courtship ritual exists in which the male displayed the tarsomere of mid leg before making attempts of genital contact [7].

***Themira lucida*** (Stæger, 1844)

Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: Sepsidae, Subfamily: Sepsinae, Genus: Themira, Species: *Themira lucida*

The main diagnostic features of the adult is having a postpronatal seta and a pruinose katepisternum. The adult fly is best identified by completely dark legs, glossy scutum and in the males by the structures on the forelegs and genitalia [2].

The adults, the head is mostly black in colour with a dull brownish colouring on parafacial, face and gena. The antenna, thorax, scutellum, abdomen and legs are entirely black [2]. The males lack the short but expanded posterior seta on mid tarsomere 4 that is present in *T. minor* and is the only distinguishing feature between the two species. It is found in most areas of Europe but more to the middle and southern part of Europe [2].

The adults are found in and around tarns and pools, damp meadows and on the river banks [8]. They breed on dung, especially goose and duck droppings [9] but are also found to breed on rabbit droppings [10]. The adults have been known to carry two unidentified species of mites (Parasitidae).

## 2.2 Growing and maintaining the flies

Our first priority was to set up cultures of the two species under study, to provide us with a supply of flies for our experiments. During the course of this project, we were required to maintain our own cultures. The two cultures were grown in plastic containers which contained:

1. A piece of damp cotton gauze to maintain the moisture in the containers

2. A damp cotton strip that is soaked in sugar-water to provide food for the flies.

3. Dung, placed in a Petri dish for the flies to lay their eggs, as well as an additional source of food. *T. minor* was grown on cowdung, while *T. lucida* needed goosedung.

The containers were then placed inside an incubator, set at 70% humidity and $25^o$C temperature, which is similar to the fly's natural habitat. The incubator lights were set on a cycle, eight hours of light and twelve hours of night with 2 two hour settings of medium light. All this was done in the hope of stimulating the flies to act as much like they would act in the wild as possible.

For each species, we began with a single container containing that species, which we called the 'parental culture'. The parental culture was allowed to grow by itself, with only regular spraying of the cotton with water to ensure continued humidity, as well as fortnightly replacing of the dung so that it would not grow stale.

After a generation, some of the dung from the parental culture was removed and examined to check if maggots were present. If so, this dung was placed in a new container, which we called the 'experimental culture'. Within two to three days, the maggots would metamorphise into juvenile flies. Before they became sexual mature, these juvenile flies would be seperated into two other containers on the basis of their sex. Those containers were identical to the ones described above. This step ensured that all the flies tested were virgins at the time of the experiment.

## 2.3 Experimental protocol

About a week or so after the preparation of the experimental cultures (once the flies had matured), actual experimentation could begin. The experiment involved observing the behaviour of the flies interacting with one another using the microscope, while simultaneously recording it on a VCR recorder. The activities of the flies were recorded as well, for reference in the future.

When performing a replicate, we would begin by flipping a coin to decide whether to observe two males, two females, or one male and one female. These procedures are described below.

### 2.3.1 Interactions between a male and a female

While we were observing male-female interactions, we would take two flies in a Petri dish and place them under the microscope. The flies would be observed for a minimum of ten minutes. If the male had not mounted the female within this time, we would put them aside, keeping an eye on them while we performed another replicate (we noticed that if nothing happened in the first ten minutes, nothing was likely to happen no matter how long we waited). Once the male mounted, we would zoom in on the pair in order to better observe their actions. We had to continuously move the microscope around, as the microscope could only focus on one area of the Petri dish at the time. In all cases, we would end our replicate within thirty minutes, unless something particularly interesting was going on.

Once the experiment was completed, we would kill the flies we had used, storing them in alcohol so that their bodies could be used for further analysis (for studying relationships between body size and mating success, for instance). The Petri dish would be thoroughly cleaned in order to remove any pheromones or other chemicals before reuse.

### 2.3.2 Interactions between males

An identical protocol to the one above was used, except that no explicit time limit was set. We continued watching until no new behaviours appeared to be occuring - in this case, once the males had started repetitively jumping on each other.

### 2.3.3 Interactions between females

Like the experiment dealing with interactions between males, we decided to impose no time limit, and merely wait until no new behaviours emerged.

## 2.4 Recording and digitization

Video records of the flies' sexual behaviour were produced using a set-up of a microscope camera, VCR and monitor. Digitization was done by using a computer, VCR and DV converter.

A Leica IC A microscope camera was integrated into a Leica MZ16A stereomicroscope and connected to a JVC SR-S990E video recorder and JVC monitor. Recordings were made on Sony Super DX tapes, each capable of recording up to 180 minutes. Video recordings were then digitized by converting analogue video recordings into digital reading using a Canopus ADVC-100 DV converter. Actual capturing of footage was done using the program Adobe Premiere 6.5. Only videos which were of good quality and relatively long durations were captured. The captured videos were then used for further analysis.

## 2.5 Evaluation

We would observed the behaviour of the flies throughout the period of the recording, while noting any interesting observable behaviours. We also did a qualitative and quantitative analysis of the behaviour of the male and females during mounting, using Adobe Premiere to view the recorded footage frame by frame.

We also did male-male and female-female experiments (see sections 2.3.2 and 2.3.3 respectively) to see what the flies' natural behaviours with other flies were (so that we could compare them with the male-female interactions), as well as observing any interesting interactions which occured when two members of the same sex are placed in close proximity.

An ethnogram was also produced, upon observing the behaviours of the flies while the male is mounted on the female, in order to quantify the interactions between two flies in this state. With the use of this ethnogram, further analysis of the sequence of behaviours displayed by the flies could be attempted.

# 3 Results

## 3.1 Interactions between a male and a female

The male-female behaviour of both species, *T. minor* and *T. lucida* , exhibit similar patterns under experimental conditions. Their precopulatory behaviour seemed pretty standardized, with only minor deviations amongst species and individual pairs. The results below are detailed for *T. minor* . Differences with *T. lucida* will be mentioned later.

When a pair of *T. minor* flies were put into the Petri dish, both the male and female fly would spend some time cleaning themselves for the first few minutes. Once this was done, both the male and female would wander about the Petri dish, circling one another. Usually the male would orientate its body in the directions of the female, sometimes stopping and rotating around in place, following the female with his eyes. The female itself would try to maintain some distance from the male and would largely ignore him, with the singular exception of wing flicks, which were observed from both flies.

During this period, the male fly would attempt to mount the female by jumping on top of her. Usually, the male would fail to mount on the first try as he did not have a proper grip on the female, who would shake him off. Pre-copulatory interaction would only fully begin when the male successful mounted the female. Once mounted, the male would clamp the female's wings with his front legs. The male front legs have appendages, found only on males, which clamp tightly onto the female's wing, preventing it from being dislodged by the female [11]. After the male had mounted correctly it would begin a set of repeated gestures. The pattern remained constant for either *T. minor* or *T. lucida* flies which were subjected to the experiment.

The pattern begins with the male extending his mid legs, moving them away from his body and in front of him, so that they are in line with the female's eyes. He then begins rhythmically curling and uncurling his tarsi (the sections of his leg furthest away from his body) and moving them towards the female in rapid jerks, bringing them almost in contact with the female's eyes before he stops. Due to its resemblance to the male sewing with a pair of large needles, we decided to name this stage **sewing**. This stage is also characterised by the male flapping his wings repeatedly and rhythmically.

The next action by the male is collectively known as **thrashing**. Thrashing can be defined as the rapid movements of the middle and hind legs, coupled with repeated flapping of the wings. The movements of the legs are random, giving the impression that the male was flailing its legs in no one particular direction. Upon closer inspection of the video tapes, however, it was revealed that the movements of the male's legs were actually a rubbing motion in which the male would extend its legs ventrally and rubbed it dorsoventrally thus rubbing the female's legs and thorax.

Following thrashing, the male will attempt to stroke the female's head. This involves the use of his middle legs to actively touch and rub against the female's head. In some cases, the female interrupted the male's stroking by beginning to stroke and clean her own head, blocking his arms.

The final set of action in the pattern is the lowering of the male's abdomen. The male would extend his abdomen posteriorly, moved rearwards to the female's abdomen. It would then rapidly tap the dorsal surface of the posterior of the female abdomen with the ventral surface of its abdomen. It would extend its genital claspers so that they touch the female's genital area and vibrate the claspers. Brushes at the end of claspers will be used to rub against the female abdomen, especially its genital area. The female almost always in rejects this action by lowering her own abdomen out of reach of the male.

This cycle of actions is repeated throughout the period of sexual interaction by the male-female pair. The average length of one cycle would be around 6 seconds. The cycle remained persistent under normal condition. The cycle would only be disrupted under abnormal condition such as the female moving towards the side of the Petri dish and then it pushing the mounted male against it, or the female fighting aggressively with the male using her midlegs. This would make the cycle difficult to be executed by the male.

On the other hand the female reaction towards the males gestures vary widely. It ranged from being passive and ignoring the mounted male to actively trying to dislodge the male. The female would shake its body sideways vigorously to try and dislodge the mounted male; at times it would use its hind leg to push the mounted male off. The female would also move around the Petri dish with the male still mounted on it. The females hind legs are stretched out when it is moving, thus it was observed that when the female moves it would drag its hind legs.

Average length of sexual interaction would last about 40-50 minutes, after which point, if there is no successful copulation, the male will dismount the female or it would be dislodged and the pair would act individually. After

| | Mounting | | No mounting |
|---|---|---|---|
| | Lead to copulation | Did not lead to copulation | Failure |
| *T. minor* | 0 | 12 | 1 |
| *T. lucida* | 1 | 7 | 1 |

Table 1: Mounting leading to copulation

being dislodged, sometime the male would try to mount the female again usually the female would aggressively shake the male off, only a few times would it allow the male to mount again.

During the course of the thirteen experiments done on *T. minor* , there were no trials that leads to a successful copulation. And out of the nine experiments done on *T. lucida* only one pair of flies leads to a successful copulation (see table 1).

During copulation the male's genitalia would be inserted into the female's, and the male and female's abdomen would be observed as interlocking with one another. During this period of time both the male and the female would be calm, the set pattern of precopulatory gestures by the males are absent during copulation. The females would still move around the Petri dish while the male copulates with it. Wing flaps were also absent from the males. The copulation lasted approximately 25 minutes and when it was over the male would separate from the female. The separation is a difficult process with both the female and moving in opposite direction while both its abdomen would still be attached to one another. Only after several attempts of pulling away from one another would the abdomen be separated. White mucus could be seen trailing of both the male and female abdomen after it was separated.

During the period of the experiment we have focused mostly on male-female behaviour of *T. minor* . Due to the time constraint we had not done any quantitative study on *T. lucida* , but qualitatively the mating behaviour for both species is extremely similar. They have the same precopulatory mating patterns and similar male-female interaction. Further qualitative and quantitative study needs to be done to compare the mating behaviour between *T. minor* and *T. lucida* .

## 3.2 Interactions between males

When two males were put together in a Petri dish, they were found to actively move around, flicking their wings at each other. The males would move around one another, occasionally orientating themselves towards each other. Sometimes, one male would stop and rotate in place, keeping himself facing towards the other male. In all four replicates tried by us, the male flies would eventually mount each other. For the sake of simplicity, the male doing the mounting will be refered to as the 'mounting male', while the male being mounted will be refered to as the 'mounted male'.

After around 3-5 minutes of actively moving around one another, one of the males would jump upon and mount the other male. He would stay on for a very short period of time, never staying on for more than five seconds in our study. When a frame-by-frame analysis was carried out after digitization, we noted that there were two types of mounting: the usual mounting procedure with both the mounted fly and the fly being mounted having their heads pointing in the same direction, and an unusual one in which the mounting fly would not orientate its body direction with that of the fly being mounted (i.e. would be facing in the wrong direction). The second type of mounting was much shorter than the first, suggesting that the fly simply didn't have the time to reorient itself.

When properly mounted on another male, the mounting male did not show thrashing and sewing behaviours (see section 4.1) as was observed when a male mounts a female. After several seconds of mounting, the mounting male would lower its abdomen such that its claspers would touch the abdomen of the fly being mounted. Prior to this action, both the mounting male and the male being mounted did not show any of the behaviours associated with males when they have mounted a female. Immediately after this lowering of its abdomen, the mounting male jumps off.

Mounting occured fairly frequently between the two observed males (our flies averaged 20 times in 10 minutes). The males would take turns to mount on each other during the period of the experiment.

There was no significant difference between *T. minor* males and *T. lucida* males in this regard.

## 3.3 Interactions between females

When two females were put in a Petri dish, it was observed that they would just walk around randomly inside the dish during the course of the experiment. The females never attempted to mount each other. However, one of the females would occasionally stop and rotate in place, keeping its head aimed at the other female (as was observed in males in both male-male and male-female interactions). The likelihood of this happening was much lesser than with males. The females would flick their wings at each other.

While the two females generally maintained a certain distance between each other, there was one recorded instance of a smaller female moving towards the larger one and turning around in place, showing it its abdomen. Whether this was intended to be some form of invitation or simply a coincidence is not known.

Interestingly, both the flies were observed to be jumping against the cover of the Petri dish, behaviour observed in neither male-male nor male-female interactions.

# 4 Discussion

## 4.1 Ethnogram

The following behaviours were noted in both sexes of flies before mounting.

- **Wing Flick**: A flicking of one or both wings, generally in the direction of another individual. Performed by both males at other males, as well as females at other females.

The following behaviours were noted in male flies when mounted on female flies:

- **Thrashing**: During this behaviour, the male rubs the female's thorax and legs. He does this erratically, in an almost random fashion, and generally also moves his hind legs in a similar fashion. The male may also rub the head of the female, beginning near the back and stroking towards her anterior end. The female might respond by rubbing her own head, thus blocking his legs.

- **Sewing**: This behaviour begins with the male holding his midlegs out on both sides of him, parallel and just above the female's eye. He then moves his legs closer and closer to the female's eyes, curling the last segment of his leg (the tarsus) in the same direction as the motion of his legs. He ends this behaviour once his legs are almost touching her eyes.

- **Lowering**: The male lowers himself backwards along the female's abdomen and touches her abdomen with his surstyli. He vibrates his surstyli against her abdomen, in what appears to be an attempt at stimulation.

The following behaviours were noted in female flies when being mounted:

- **Wrestling**: In this behaviour, the female fly uses her midlimbs to try to force the male off by pushing on his head. We have observed male flies having their head twisted upto 100 degrees by this attempt. It is generally unsuccessful, but presumably distracting for the male.

- **Moving**: In this behaviour, the female moves around the Petri dish. In the wild, she would probably be moving into vegetation to try to force the male off - by walking underneath low hanging grass, for instance. The male responds by trying to prevent her moving, by digging his hindlimbs into the ground. We have seen males force a female to stop, and sometimes even pull her backwards, just by sheer force.

## 4.2 Interactions between a male and a female

It was observed that it was the male who would initiate the mating interactions first by actively moving towards the female and orientating its body towards the female in a tracking motion that culminates in mounting. The females on the other hand would ignore the males and would try to avoid them if possible. What we see here is a behaviour that was derived from asymmetrical investment during evolution. Thus this is a part of sexual selection that will lead to unique mating procedures within a species.

In *T. minor* , the contribution towards a successful mating was unevenly distributed between male and females. This is in contrast with humans in which the contribution was relatively equal, in which the female would expend energy nurturing the child while the male would provide the food and resources needed to raise the family. In terms of energy expenditure the female flies required a substantially greater amount of energy to produce an egg as compared to the energy required to produce sperms by the males. Also during copulation itself the females occurred a greater amount of damage because the male genitalia is abundant with sharp brushes thus when inserted the female's uterus would become damaged. Thus, although the investment is asymmetrical the result is symmetrical: both flies have half their genes being passed on to the next generation.

Thus because of this asymmetrical investment the onus is on the male fly to initiate the mating because the female will remain passive. If the male remains passive it would not be able to mate and thus could not pass its genes on to the next generation. The females, on the other hand, just by remaining passive would still be copulated by one of the numerous males that would actively court it. Thus the females would preferred to focus its energy on obtaining food and dung rather than on looking for a mate to copulate with. The activity of the male could also mean an honest signal to the female to show its intention to mate with the female.

The males must put in effort to court the ever passive female so mating can succeed. Since the females are passive the males need to impress and convince the female so that it would copulate with him. This was done by precopulatory gestures initiated by the males. The vibration of the male's claspers on the female genitalia could be an attempt to sexually stimulate the female fly. The males could not force itself on the female, this was observed when the female rejected the male it would lower its abdomen preventing the males genitalia to reach it. Thus copulation could only be done with the consent of the female.

The female would prefer to mate with the best male possible thus leading to the female selecting the mounted males. When a male fly is mounted upon the female fly, most of the female fly would try to shake the male fly off by shaking its body sideways or dislodge the male fly using its legs. This vigorous behaviour is taxing in terms of energy expenditure to the females. An explanation of this behaviour is that the female is actively testing the mounted male with its aggressive behaviour in order to select the best possible mate.

The attributes selected maybe strength, endurance, symmetry and weight of the males. The shaking movements of the female fly would lead to a test of the male's endurance to hang on, whereas the pushing movements of the female's legs would test the males strength. The females could be testing the male's symmetry by looking at the sewing motion of the males mid legs and probably how constant the pattern of precopulatory cycles is.

Sometimes the situation in which a male mounted on a female could be best described as a stalemate. This was because the male is mounted so tightly on the female such that the female could not shake the male off and at the same time the female rejects the male by lowering her abdomen. When this occurrs, it is less energy consuming for the female to just give in to the male and allows copulation to take place. Does this mean that the female is testing the male, making sure that he is fit before letting him copulate? Or is there some hidden cost to reproduction which we don't know about yet?

More research is needed before we can be sure.

Although most of the study has been focused on *T. minor* , *T. lucida* shows significant similarity with *T. minor* . Qualitatively they show strikingly similar behaviour, although it had not been quantitatively described. There was a circumstantial observation that the cycle for the *T. lucida* occurs at a faster rate as compared to *T. minor* but further quantitative data needs to be obtained to confirm this which could not be obtained due to the lack of time.

In one spontaneous trial we put in a male *T. minor* with a female *T. lucida* and interestingly the male would not attempt to approach or jump at the female it would just look at it. We added two more *T. minor* but all the male flies would not jump at the female. When a male *T. lucida* was added almost immediately the male *T. lucida* would jump and mount the female *T. lucida* . This would seem that the male fly could tell that female fly belongs to different species that is morphologically very similar but it could not tell between a male and female of the same species. Most probably visual cues are not used to differentiate between species because of the extreme similarity between the two species, we could presume that some sort of pheromones could be used to differentiate between the two species, further study needs to be done to confirm this.

## 4.3 Interactions between males

*T. minor* males exhibit an interesting behaviour, the males of the same species would jump to another male even after a period of time in which they would move around one another and actively looking towards one another. It was clearly observed that the males would actively orientate their bodies towards another male. The males seemed to be tracking one another; after tracking they would still jump at the other male. This would mean that some other form of mate recognition other than visual cues must have been used by the flies. Other mean of sexual differentiation must be implemented by the male flies be it by other sensory cues such as touch or pheromones. Since in the laboratory we have limited resources thus we could not investigate mating cues via pheromones, further research could be done to elaborate on this aspect.

An interesting observation was that after the male mounts another male it would not initiate the set-sequence observed in mounting a female. The male fly would just hold on the other and only after a few seconds would it lowered its abdomen. It would use its claspers to touch the abdomen of the fly being mounted. This action seemed a form of an 'honest signal' to confirm the sex of the fly that it is mounting. It seemed that the mounted male was already suspicious that the fly that it has mounted was not of the opposite sex, thus it did not initiate the precopulatory mating actions as it would have when it mounts a female fly. However, any cues that would inform the mounted male fly that the fly that it was mounting is not a female prior to lowering of its abdomen was not observed. We could not rule out that there might be pheromones used as a cue which could lead to the mounted male fly not initiating its normal precopulatory mating actions.

That males being active even towards one another because they could not differentiate sexes by visual cues was quite expected. With the females being naturally passive the males were the ones that initiates the mating process. Thus it is natural for the males to try and copulate with any fly of the same species irregardless of the sex because if the did not try they would not be able to pass on their genes, whereas for the female flies even with being passive it would eventually be copulated by a male.

## 4.4 Interactions between females

The females being naturally passive and asocial shows little behaviour and communication when it was paired with another female. Usually both the females seemed to be sexually uninterested in one another and thus only walked around randomly and occasionally looking at one another because of general interest. The two females usually maintained a distance between them. The isolated instance in which one female touted its abdomen towards another might be the case in which since the fly is virgin it might want to get copulated quickly. We have not done any comparison study between a mated female fly and a virgin fly hence we could not rule out whether the inclination of a female fly to get copulated quickly is related to whether the female fly is still a virgin.

The observed action in which the two female flies seemed to want to get out of the Petri dish might be due to the absence of any food and dung in the Petri dish. The female although it is passive it would eventually be copulated by a male. Thus it has no worries of not being able to pass its genes to the next generation, therefore the female fly is more concerned about obtaining food and dung and when no food or dung was present both the female flies wanted to leave the vicinity.

## 4.5 Difficulties in this project

During the course of the investigation and experimentation, a number of show-stopping difficulties arose in the cultivation of the fly cultures as well as the recording of the fly sexual behaviour.

A major problem that developed was the sudden and re-emerging infestation of ants into the fly cultures. These ants would, in under a day, manage to prey on the flies and almost decimate the population. It is still unknown exactly what attracts the ants to the cultures and how they manage to invade the plastic containers, though several speculations can be made.

Yet, due to the infestation, population of both species suffered large drops. We were forced to stop experimentation and to merge the experimental cultures back with the original cultures so as to have the largest number of reproducing adults in the cultures, to quickly regenerate the population back to pre-ant values. The ants invaded repeatedly for several weeks, attacking several cultures, making it impossible for us to properly quantify the sexual behaviour in *T. lucida* .

# 5 Conclusion

At the end of this study, we find the precopulatory behaviour of Sepsid flies to be more interesting then previously thought. For a sepsid fly, sight is not enough to determine sexuality, instead, other methods of communication take precedence. At the level of insects, there are such layers of complexity involved in sexual activity which suggest that higher on the evolutionary scale, much more complexities are involved in the sociological aspects of mating. One need only look at the activities of humans to realize that sexual interaction is a large field in behavioural evolution that bears closer inspection in order to further understand the nature of evolution and where it will take us.

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