Evaluation of Mating Behaviour and Mating Compatibility Methods for the Old World Screwworm Fly, *Chrysomya bezziana*

Wardhana AH[1], Cameron MM[2], Muharsini S[1], Hall MJR[3]

[1] *Indonesian Research Centre for Veterinary Science, P O Box 151, 16114, Bogor, Indonesia*
E-mail: wardhana24id@yahoo.com
[2] *Department of Disease Control, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, U. K.*
[3] *Life Sciences Department, Natural History Museum, London SW7 5BD, U. K.*

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ABSTRACT

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The effectiveness of the Sterile Insect Technique program (SIT) to eradicate pest insects relies on the success of mating competitiveness between irradiated male flies and wild type males for the wild type females. It has been successfully applied for the New World screwworm fly (NWSF), *Cochliomyia hominivorax* but remains unproven for the Old World screwworm fly (OWSF), *Chrysomya bezziana.* The aim of the study was to develop methods for investigating mating behaviour and mating compatibility of *C. bezziana* under laboratory conditions. Two methods were used for studying mating: individual mating (method 1) and group mating (method 2). The flies used in this study were 5-7 days old. Twenty four hours after emergence, adult flies were sexed and placed into different cages until studied. The female : male ratio in the group mating was 1 : 5 and the males were marked by painting a dot on the thorax using different oil colours. Observation of mating behaviour was investigated every 30 minutes through 10-20 replications for all methods depending on the availability of flies. Data were analysed using ANOVA and the Student's t-test, with significance demonstrated at the 95% confidence level. The results demonstrated that the frequency of contacts between males and females at different ages was a significantly different ($p < 0.05$) and that the duration of copulation was not significantly affected by fly age both method 1 ($p > 0.05$) and method 2 ($p > 0.05$). Copulation was only initiated following longer periods of contact, mainly in the range of 270-449 seconds. The highest frequency of copulation occurred between 7-8 days, but the duration of mating was similar between 5-8 days old. The study demonstrated that the methods developed were suitable for a mating compatibility study of *C. bezziana.*

Key Words: *Chrysomya bezziana*, Mating Behaviour, Copulation, Myiasis

ABSTRAK

Wardhana AH, Cameron MM, Muharsini S, Hall MJR. 2013. Evaluasi perilaku kawin dan metode kecocokan untuk kawin pada lalat the Old World Screwworm Fly, *Chrysomya bezziana.* JITV 18(4): 265-273. DOI: 10.14334/jitv.v18i4.333.

Keefektifan program Sterile Insect Technique program (SIT) atau teknik pemandulan insekta untuk memberantas hama tergantung pada keberhasilan kompetisi antara lalat jantan yang diiradiasi dengan lalat jantan di alam (liar) untuk kawin dengan betina di alam (liar). Program ini telah sukses diaplikasikan untuk memberantas the new world screwworm fly (NWSF), *Cochliomyia hominivorax* meskipun masih menyisakan pertanyaan untuk lalat myiasis yang lain, yaitu the old world screwworm fly (OWSF), *Chrysomya bezziana.* Tujuan penelitian ini adalah untuk mengembangkan metode yang digunakan dalam pengamatan kecocokan pasangan dan perilaku kawin lalat *C. bezziana* pada kondisi laboratorium. Dua metode kawin dikembangkan, yaitu kawin secara individu (metode 1) dan kelompok (metode 2). Umur lalat yang digunakan pada studi ini adalah 5-8 hari. Setelah 24 jam pasca menetas, lalat dipisahkan antara jantan dan betina dan masing-masing dipelihara pada kandang yang terpisah hingga digunakan untuk perlakuan uji. Rasio betina dan jantan pada metode kawin kelompok adalah 1 : 5 dan lalat jantan diberi tanda titik pada toraknya menggunakan cat minyak. Perilaku kawin lalat diamati setiap 30 menit dengan ulangan 10-20 tergantung pada ketersediaan lalat di laboratorium. Data yang diperoleh dianalisis dengan ANOVA dan Student's t-test pada tingkat kepercayaan 95%. Hasil penelitian menunjukkan bahwa terdapat perbedaan frekuensi kontak antara lalat jantan dan betina yang nyata antara umur 5 hingga 8 hari ($p < 0,05$) meskipun tidak terdapat perbedaan yang nyata pada lama waktu kopulasi baik dalam metode 1 ($p > 0,05$) maupun metode 2 ($p > 0,05$). Kopulasi hanya terjadi apabila lalat jantan dan betina kontak dalam waktu yang lebih lama antara 270-499 detik. Frekuensi kopulasi yang tertinggi terjadi pada lalat yang berumur 7-8 hari, walaupun durasi kawin antara lalat umur 5-8 hari relative sama. Hasil penelitian mengindikasikan bahwa metode kawin yang dikembangkan dalam studi ini dapat diaplikasikan untuk studi kecocokan kawin pada lalat *C. bezziana.*

Kata Kunci: *Chrysomya bezziana*, Tingkah Laku Kawin, Kopulasi, Myiasis

INTRODUCTION

The larvae of the Old World screwworm fly (OWSF), *Chrysomya bezziana* (Diptera: Calliphoridae), cause myiasis of vertebrate animal and remain a major problem over African and Asian regions, including the archipelago country of Indonesia. Many efforts to control or prevent screwworm myiasis have been applied, but they have produced mixed results. The Sterile Insect Technique (SIT) is a proven method of control leading to eradication pest insects (Vreysen, 2005). The technique was successfully applied to eradicate a primary myiasis agent, the New World Screwworm fly (NWSF), *Cochliomya hominivorax*, from the North American continent and more recently from Libya and from most countries of Central America (Lindquist & Abusowa 1992; Baumhover et al. 2000; Whitten 2002; Dyck et al. 2005). More recently, Panama was declared as a screwworm-free region in 2006 and a buffer zone of 30,000km^2 was established at the Darien Gap to inhibit reinfestation by the weekly release of 25-50 million sterile males (Garcia et al. 2007).

The SIT has been effective not only against the NWSF, but also against the Meditteranean fruit fly (*Ceratitis capitata*) (Hendrichs 2000), the tsetse fly (*Glossina* spp) (Vreysen 2001), the melon fly (*Bactrocera cucurbitae*), the Queensland fruit fly (*Bactrocera tryony*), the Mexican fruit fly (*Anastrepha ludens*), and the West Indian fruit fly (*Ananstrepha oblique*) (Cayol et al. 2002).

One fundamental requirement for the success of eradication programmes using the SIT is that irradiated male flies must compete equally against the wild males for mating with the wild females (Mayer et al. 1998). Lance & McInnis (2005) defined the mating competitiveness of sterile males as a function of their mating propensity and mating compatibility. Mating propensity is the tendency to locate a mate, copulate and inseminate, while the mating compatibility is a relative measure of how readily two populations of insects are reproductively compatible.

Most mating compatibility studies on screwworm flies referred to mating of sterile males with wild females (Spradbery et al. 1983; Mayer et al. 1998). Taylor et al. (1991) assessed the reproductive compatibility between *C. hominivorax* from North Africa (Libyan population) and the Central American strain that was used in SIT releases, produced in Chiapas, Mexico but originally collected in Orange Walk, Belize. While the two strains examined were genetically different, based on *mt*-DNA restriction site analysis, the study demonstrated that the North African strain was compatible with the Central American strain and that there was no mating barrier between them. This provided strong support to the use to the Belize strain could be used to eradicate *C. hominivorax* in North Africa. Similarly, Mastrangelo et al. (2012) also demonstrated that mating barriers would not compromise the use of Jamaican sterile males for the SIT campaigns against *C. hominivorax* in Brazil.

In view of the geography of the Indonesian archipelago, which has thousands of islands, the mating compatibility of *C. bezziana* populations on the different islands is a cause of great concern before considering whether the SIT programme should be applied or not. Compatibility assays will be a particular priority for those screwworm populations which are shown to be genetically distinct (Wardhana et al. 2012). For example, the numbers of myiasis cases due to screwworm in Java and Sumatra islands are high, but there is a clear overall genetic distinction between populations on the islands. These islands are separated by the Sunda Straits which could act as a reproductive barrier (geographical isolation). Therefore, studies on the mating compatibility of *C. bezziana* populations in Indonesia should start by comparing populations from Java and Sumatra (Wardhana et al. 2012).

Unlike the well documented mating compatibility studies between *C. hominivorax* strains, studies on mating behaviour, mating competitiveness, mating preference and reproductive compatibility of *C. bezziana* are very rare (Garcia, 2002; Mastrangelo et al. 2012). Accordingly, the aim of this study was to develop methods for investigating mating behaviour and mating compatibility between populations of *C. bezziana* that were genetically identified as belonging to different *cyt b* haplotypes and lineages (Wardhana et al. 2012).

MATERIALS AND METHODS

Chrysomya bezziana sampel

Flies from the laboratory colony of *C. bezziana* (394th generation) from the Department of Parasitology, Indonesian Research Centre for Veterinary Science (IRCVS) Bogor were used in this study. The larval and rearing procedures for *C. bezziana* followed the guidelines of Sukarsih et al. (2000), with a slight modification in the larval rearing media (LRM), where the dried blood and water lock gel were replaced by fresh bovine blood and CF100, respectively (Table 1).

Table 1. Ingredients of modified LRM

Ingredients	Amount	Supplier
Fresh bovine blood	300 g	Local
Low-fat milk powder	30 g	Local
Whole egg powder	30 g	Sunny Queen Products, Brisbane, Australia
CF 100	50 g	Grain Processing Corp. Muscaline
Formalin 10 %	1 ml	AR Grade
Distilled water	980 ml	Local

Medias for rearing of *C. bezziana* larvae

Meat-blood mixture media (MBM)

Lean minced beef (250 g) and fresh beef blood (30 ml) were homogenized in a blender. This media was prepared for larvae that had just emerged from egg masses (L1) (Sukarsih et al. 2000).

Larval rearing media (LRM)

New ingredients of the modified LRM from the recipe described by Sukarsih et al. (2000) recipe are shown in Table 2. Fresh bovine blood, low fat milk powder, whole egg powder, 10% formalin and distilled water were homogenized using a blender. CF 100 was added and mixed gently. This media was prepared for 2nd and 3rd instar larvae.

Larval and fly rearing procedure

To rear *C. bezziana* colonies using artificial media, at least two rooms are required; larval and adult fly rooms. For the larval room, the temperature and relative humidity were controlled at 30-32°C and 75-80%, respectively, and with low light intensity. For the adult fly room an uncontrolled ambient room temperature was used, with the same relative humidity (75-80% RH) and the room was darkened by placing UV car window film (40% light transmission) over the windows. Each room was fitted with an extraction fan to vent the odour of the rearing medias to the outside and all holes in the room were covered by gauze to prevent both the escape of flies and the entry of flies from outside (Sukarsih et al. 2000).

A 125 mg egg mass was deposited onto 50 g, MBM placed in the corner of a plastic hatching tray (18.5 x 13.5 x 4.5 cm deep). A small amount of fresh LRM was added to the tray just around the MBM. The tray was then covered with gauze and then a damp towel was placed over half of the gauze, over the eggs, to maintain the relative humidity. This tray was put on an electric blanket and incubated overnight at 37°C. Within 8-10 hours, the 1st instar larvae (L1) hatched and naturally migrated from the MBM to the LRM. Twenty hours after hatching, the fresh LRM was added to fill the tray and incubated overnight. When the larvae moulted to L2, the LRM containing the larvae was transferred from the hatching tray to a clean larval rearing tray (30 x 23 x 4.5 cm). Fresh LRM (1400 g) was added to fill the tray and larvae were incubated therein for two days. The gauze and damp towel covers were not required anymore. Larvae were incubated in this media for 2 days. After five days, the larvae escaped from the LRM to pupate in the vermiculite on the floor of the container and they emerged as adult flies after being incubated for about seven days.

Sexing newly emerged adults

Within 24 hours of emergence, adult flies were removed from the big screen cages in which they emerged (45 x 30 x 25 cm) using a glass tube. They were then sexed and transferred into small single-sex small screen cages (30 x 30 x 30 cm) for use in the preliminary study of mating behaviour and mating compatibility. Each sex (female and male) was held separately in the small screen cages in groups of up to 50 flies per cage (Spradbery 1988; Lance et al. 2000). The emerged flies were held in the larval rearing room (at 30-32°C and 75-80% RH) for two days while waiting for all adults from a batch to complete emergence. On day 3, the flies were moved to the adult room where they were fed with lean beef meat and a small drop of fresh blood, water and sugar lumps until the fourth day. When they were aged 5 days, they were fed only with water and sugar lumps (Sukarsih et al. 2000).

Marking of male flies

For both individual and group assays, experiments involved five males per replication representing different populations. Male flies were collected from the cage using a glass tube and put into a freezer

(-20^{o}C) for 2.5 minutes. After cold immobilization, they were put into a Petri dish and marked by painting a dot on the thorax with different oil colours to enable distinguishing one from each another (Lance et al. 2000). Those flies with the same colour were held in the same cages, so that five cages were required for the different colours (red, yellow, blue, white and green).

The effect of marking on male flies was investigated to determine whether the marked male flies could be directly used in the assay or if they should be held for 24 hours after marking to give them a chance for adaptation to the procedure.

Observations were carried out by comparing the behaviour of marked flies and unmarked flies for 30 minutes. Thirty male flies were randomly captured from the small screen cage using a glass tube and they were divided into two groups, marked and unmarked groups. Individual fly from each group was put into an individual jar (12 cm length x 6 cm diameter) which was covered by gauze. Observations were performed on day zero (the day when the flies were marked) and on day one (one day after marking). Their behaviours were scored into 5 categories:

Score 0 : The male fly did not move, it just stood still on the inside surface of the jar, i.e. zero response
Score 1 : The anterior legs of the fly were rubbed together or used to clean the fly's face
Score 2 : The male tried to clean its wings using its posterior legs
Score 3 : The posterior legs of the fly were rubbed together
Score 4 : The male fly moved around inside the jar

Observation of mating behaviour

Observation was made to determine the duration of the period of courtship of a female and male pair, from initiation until they completed copulation. Flies of different ages (5, 6, 7 and 8-day old) were tested to identify the optimum age for undertaking observations of mating behaviour. Five marked males were placed into a glass jar (12 cm length x 6 cm diameter) together with 1 female. Observation of mating behaviour was carried out in all experiments for 4 hours and mating encounters were scored into 5 categories:

Score 0 : There was no interaction between a male and the female. They stayed apart from each other.
Score 1 : A fly approached another fly of the opposite sex. There is courtship behaviour between a male and the female but the male did not mount the female.
Score 2 : A male mounted the female but in the wrong orientation.
Score 3 : A male mounted the female in the right orientation.
Score 4 : A male was successful in copulating with the female (i.e. genitalia engaged).

Mating protocol

Two methods of mating for *C. bezziana* were evaluated in this study defined as method 1 (individual mating) and 2 (group mating).

Method 1 (individual mating)

One female and one marked male (with any colour) were captured from the small screen cage using a glass tube. They were paired in a glass jar (12 cm length x 6 cm diameter) to enable the visualization of courtship and mating. The mating behaviour was observed for 30 minutes with 10 replicates.

Method 2 (group mating)

This experiment used five males each with a different mark on the thorax, which in a true mating compatibility study would have represented a different population to the female or, potentially, different male populations. However, in this trial males and females were all from the same populations (Bogor laboratory colony). Method 2 was used to assess mating compatibility and was replicated 10-20 times.

Using a glass tube, one male was captured from each of the different colours of small screen cages and put into the observation jar. When all five marked males were ready, one female were released into the jar. Observations of mating compatibility and their mating behaviour were recorded for 30 minutes and scored based on the five categories outlined above (scoring of mating behaviour).

Statistical analysis

Statistical analysis of mating behaviour and mating compatibility was carried out using SPSS version 17 for Analysis of Variance (ANOVA) and the Student's t-test with significance demonstrated at the at 95% confidence level.

RESULTS AND DISCUSSION

Mating behaviour of *C. bezziana*

To date, there is no published study on mating behaviour of different populations of *C. bezziana*. A small hybridization study using *C. bezziana* was conducted by J. P. Spradbery in the United Kingdom

(1988 unpublished) where flies from Malaysian, Papua New Guinea, Indonesian, Oman and South African populations were crossed. His findings demonstrated that, among those populations tested, South African females were reluctant to oviposit under laboratory conditions (6/79 females) even though they were successful in copulating. The study was conducted until the generation F1, so the rate of sterility of hybridized flies could not be evaluated. In addition, the study did not record when the fly pairs started to copulate, the best age for mating nor the behaviour of the flies during mating.

According to Wicker-Thomas (2007), the courtship behaviours of the order Diptera are very diverse and involve sex pheromones in the process of mating. However, often pheromones are only one of the many signals emitted by the insect and in most groups of dipterans, the functionally active components are not known. The present study evaluated two methods to observe the mating behaviour of *C. bezziana* under laboratory conditions.

Ideally, the preliminary study of mating behaviour assay used 20 replicates (Spradbery 1988). However, due to the limited number of flies available for study, the assays performed here used various numbers of replicates, from 10 to 20. Another problem was that, the number of females and males obtained from the experimental rearing was not the same. Nevertheless, this study still produced some novel and interesting results which could serve as a baseline for further studies.

Effect of marking on male flies

Marking male flies using paint spots on the thorax of significantly flies influenced their behaviour compared to unmarked, control flies only on the day of marking (day zero) ($p < 0.05$). However, one day after colouring, the behaviour of the same male flies did not differ significantly from the controls ($p > 0.05$) (Figure 1).

On day of marking, marked flies rubbed their anterior legs (score 1) more frequently (30.87 times) than unmarked (control) flies (16.27 times) and they also more often tried to clean (score 2) their wings using their posterior legs (38.53 times for marked flies and 17.27 times for unmarked flies). However, the study showed that the behaviour of marked flies returned to normal within 24 hours of marking (Figure 1). Therefore, it is recommended that future studies using paint marked flies in behavioural studies use them only after a 24-hour period of adaptation.

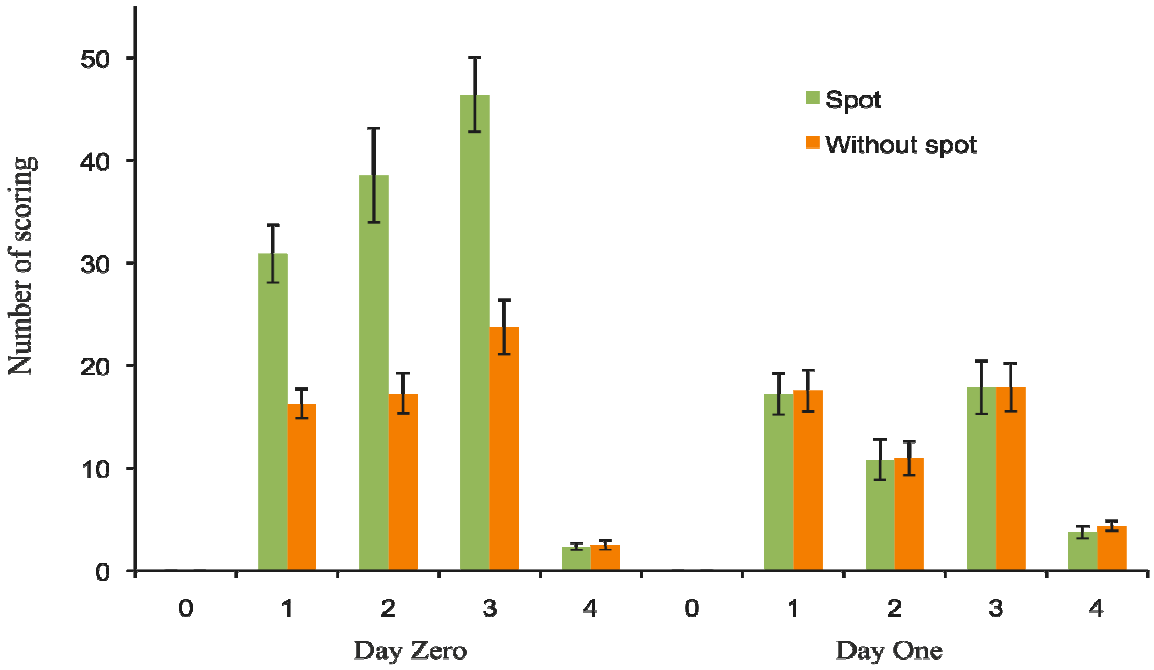

Figure 1. Effect of marking on the behaviour of male flies compared to unmarked controls on the day of marking (day zero) and one day after marking (day one) Scores were totaled over a 30 minutes period (n=15 replicates)

Observation of mating behaviour on individual mating (Method 1)

Observation of contact period between males and females was scored to investigate mating behaviour of *C. bezziana* in different ages. For individual mating encounters, the assay was conducted on flies aged 7 and 8 days old only due to limited number of flies at the laboratory. The result showed that the frequency of successful copulation (score 4) of 7 days old pair of flies was 40% whilst 100% for 8 days old. Of the 7 days old flies, 40% showed no interaction between male and female in the jar (score 0) throughout the entire observation time (30 minutes). The proportion of flies that initiated mating encounters within the time period 0'00"-2'59" from the start of the experiment was 30% and 100% for 7 days old and 8 days old flies, respectively.

Observation of mating behaviour in group mating (Method 2)

Like individual mating, a five-level system of scoring mating activity was applied to observe mating behaviour of *C. bezziana* by method 2 (group mating). The results showed that the most frequent score recorded was 3 (the male mounted the female in the right direction for copulating) in all age groups (62.34-77.9%), followed by score 2 (the male mounted the female but in the wrong direction for copulating) (Table 2). There was a significantly difference in the number of contacts between male and the female at different age of *C. bezziana* ($p < 0.05$), with he flies 8 days old flies being more active (Table 2).

The duration of contact between male:female pairs was related to the degree of mating activity and whether or not copulation was achieved (Table 3). Short contacts of 0 - 89 seconds duration never involved full copulation. Copulation only occurred with longer periods of contact, mainly in the range of 270 - 449 seconds. However, some fly pairs showed full copulatory responses during encounters of just 90 - 210 seconds duration. The highest frequency of contacts for all ages, but especially for flies aged 7 and 8 days old, were those of less than 30 seconds.

The proportion of successful copulations in this assay was 55-80% for flies aged 7-8 days. The 5 days old flies showed a low response, with just 40% of trials ending in successful mating. Most full copulation encounters started within 6 minutes of the introduction of the female into the jar at the start of the trial. Some trials required a longer time to initiate full copulation (> 9'00"). The longest interval between start of the trial and start of copulation was 27'00"-29'59" observed in one of the trials with the 7 days old flies (Table 4).

Under laboratory conditions, the durations of copulatory encounters of *C. bezziana* using methods 2 and 1 were very similar (Table 5). Flies of 7-8 days old copulated for 290.25-352.60 seconds during the individual mating method (method 1) and 267.82-353.50 seconds during the group mating method (method 2). Based on statistical analysis, the duration of copulation among ages of flies was not significantly related to fly age both in method 1 ($p > 0.05$) and method 2 ($p > 0.05$).

Previously, the peak of mating of screwworm fly (the NWSF) was reported to occur at 5 days after emergence for females and males (Crystal 1967; Adams 1979). This result was also observed when 700 flies which were put in the screen cages in laboratory (untreated flies) (Sukarsih et al. 2000). However, the observation reported here for 30 minutes in the experimental jar (method 2) showed that the highest frequency of copulation occurred among 7-8 days old flies. Flies of 5 days old were reluctant to mate even when observed for 4 hours.

Table 2. Distribution frequency of mating behaviours at different age of *C. bezziana* observed for 30 minutes

Scoring	Frequency of scoring at different age (f, %)			
	5 days	6 days	7 days	8 days
0	0 (0,00)	0 (0,00)	0 (0,00)	0 (0,00)
1	4 (8,89)	1 (1,30)	5 (4,00)	4 (4,21)
2	6 (13,33)	15 (19,48)	12 (9,60)	9 (9,47)
3	31 (68,89)	48 (62,34)	95 (76,00)	74 (77,90)
4	4 (8,89)	13 (16,88)	13 (10,40)	8 (8,42)
Total	45 (100,00)	77 (100,00)	125 (100,00)	95 (100,00)
n	10	20	20	10
Means of contact	4,5	3,85	6,25	9,5

Table 3. Comparison between the duration of copulating (C) and not copulating (NC) encounters between fly pairs investigated in the experimental jar using method 2 (group mating) over a 30 minutes period. Encounters were pooled into intervals of 30 minutes

Period of contacts at interval of 30 seconds (seconds)	Ages of flies (days)							
	5		6		7		8	
	C	NC	C	NC	C	NC	C	NC
0 - 29		32		37		93		63
30 - 59		6		17		14		8
60 - 89				5		3		7
90 - 119			1	2	1	3		1
120 - 149			1	1	1	1		4
150 - 179				1				
180 - 209				1	2			1
210 - 239							1	
240 - 269			2		2			
270 - 299	1		4				1	1
300 - 329	1		1				1	
330 - 359	1		1		2			2
360 - 389			2		2		1	
390 - 419			1		1		2	
420 - 449	1						2	

Table 4. The effect of fly age on the time of initiation of full copulation within observation jars (one female with five males) and the proportion (%) of trials with a full copulatory response

Time to start mating	Frequency of mating at different age of flies			
	5 days	6 days	7 days	8 days
0'00"-2'59"	2	6	4	6
3'00"-5'59"		1	4	1
6'00"-8'59"				
9'00"-11'59"		1	2	
12'00"-14'59"	1	2		
15'00"-17'59"	1	1		1
18'00"-20'59"				
21'00"-23'59"		2		
24'00"-26'59"				
27'00"-29'59"			1	
Total	4 (n-10)	13 (n-20)	11 (n-20)	8 (n-10)
Proportion (%)	40	65	55	80

Table 5. Comparison of durations of copulatory (mean ± SE) encounters between method 1 (individual mating) and method 2 (group mating) at different fly ages

Methods	Fly age	Means ± SE (seconds)
Individual mating (Method 1)	7 days	290.25 ± 99.73
	8 days	352.60 ± 31.82
(Group mating) Method 2	5 days	339.50 ± 31.45
	6 days	280.31 ± 13.44
	7 days	267.82 ± 17.96
	8 days	353.50 ± 17.67

This result on actual copulation activity was in agreement with results from the investigation of general activity of flies in the observation jar, which showed that flies 5-6 days old were less active than those that were 7-8 days old.

In the context of the SIT, according to resource distribution, Thornhill & Alcock (1983) divided the types of male mating systems into three categories: the resource-defense polygyny, prolonged searching polygyny and lack polygyny. In the first category, the potential for mate monopolization by males is high due to a small aggregated distribution of females. In this case, male mating success is predominantly determined by intra-sexual competition at the resources required by females and a rapid sexual response is optimal. In the second category, mating takes place away from resources required by females and in the third category, the potential for males to monopolize resources and female is rather low. *Chrysomya bezziana* was characterized in the first category, where males seek females at wounds on host animals (i.e. at resources required by females) and the interaction between the sexes is relatively simple and quick, as soon as they meet. The present study demonstrated that flies start mating soon after coming into close vicinity with each other, generally within 3 - 6 minutes after they were introduced into the experimental jar.

Comparison of the duration of mating (copulation) between methods 1 and 2 gave similar result. For flies 7-8 days old, the duration of mating was found to be 267.82 - 353.50 seconds. Regrettably, there were no flies 5 - 6 days old for method 1 assay due to limited availability of lies. However, the duration of mating recorded using method 2 for flies aged 5-6 days old was in the same range as flies aged 7-8 days old (i.e. 280.31-339.50 seconds), demonstrating that the duration of mating was similar regardless of age between 5 and 8 days.

CONCLUSION

Mating compatibility studies on *C. bezziana* were conducted by two methods, individual and group matings. Full copulation only occurred with longer periods of contact between male and female, mainly in the range 270-449 seconds. In addition, the highest frequency of copulation occurred between 7-8 days old and the duration of mating of flies was similar between 5-8 days old. The methods used here are suitable for studies of mating compatibility. Further observation would be required to investigate survivability rate of the hybridized flies.

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