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ORIGINAL ARTICLE

Effect of Age and Multiple Mating on the Mating Behaviour of *Forcipomyia* sp. (Diptera: Ceratopogonidae) Found in the Rohilkhand Terai Region

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ABSTRACT

The mating behavior of the biting midge Forcipomyia sp may be divided into a number of stages, including resistance behavior of the female before copulation. A previous study indicated that resistance behavior is a method through which the female assesses the fitness of the male, allowing some degree of mate choice. Our study used behavioral to investigate whether insect age and the reproductive status of the females influenced mating behavior. Virgin females became increasingly receptive to mating attempts as they aged, with reduced resistive behavior and a reduction in the time required for genital union. With increasing male age, the duration of copulation increased, possibly related to the time required for the transfer of sperm. Previously mated females were very resistant to further attempts to mate by males. Secondary matings were achieved only through the persistent efforts of males, although when successful, there were no differences in the duration of the mating process from matings with virgin females. Virgin female Forcipomyia sp release a volatile sex pheromone which is detected by the males' antennae. which may allow males to distinguish virgin from mated females. Our laboratory trials showed that as virgin females get older, they become far more welcoming to male courtship attempts. This shift was marked by a sharp drop in their defensive movements and a much faster path to structural alignment. On the flip side, we noticed that older males took significantly longer to finish copulating, which most likely points to a slower, age-related mechanical delivery of sperm. When we looked at females that had already mated once, they fought back aggressively against any secondary males trying to court them. These persistent males could only force a secondary pairing through continuous, high-energy effort; though once they managed to connect, the physical duration of the actual mating event looked identical to pairings with virgins. These findings provide insight into mate choice mechanisms, reproductive behavior, and pheromonal communication in Forcipomyia sp., with possible implications for understanding population dynamics and vector management strategies in the Rohilkhand Terai region.

Keywords: Forcipomyia *sp., female resistance, multiple mating, chronological age, sex pheromones, swarm density, Rohilkhand Terai, vector control*

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INTRODUCTION

Biting midges belonging to the genus *Forcipomyia* (Diptera: Ceratopogonidae) are small but ecologically important insects commonly found in moist and semi-aquatic habitats. These insects exhibit complex reproductive and mating behaviors that are influenced by several biological and environmental factors. Understanding the mating system of *Forcipomyia* species is important for explaining their reproductive success, population maintenance, and behavioral adaptations under natural conditions. In the Rohilkhand

Terai region of Uttar Pradesh, favorable climatic conditions such as high humidity, dense vegetation, and availability of breeding sites support the abundance and seasonal activity of these midges.

Mating in *Forcipomyia* sp. generally occurs during specific periods of the day when males aggregate in swarms near visual markers and compete for approaching females. Swarm formation plays an important role in mate encounter and successful copulation. Within these swarms, body size, physiological maturity, flight activity, and physical fitness influence male competitiveness. Larger and more active males are often more successful in securing mating opportunities than weaker individuals. Such behavioral interactions contribute significantly to mate selection and reproductive fitness in natural populations.

The mating sequence in *Forcipomyia* sp. includes a distinct pre-copulatory phase during which females display resistance behavior before accepting males for copulation. This resistance is considered an important mechanism of mate assessment, allowing females to evaluate male quality. Female age strongly influences this behavior. Young virgin females usually exhibit greater resistance, whereas older virgin females become more receptive to mating attempts, resulting in reduced defensive movements and faster genital union. Male age also affects mating performance, as older males generally require a longer copulation period, possibly due to reduced sperm transfer efficiency.

Female reproductive status is another important factor affecting mating behavior. Previously mated females show strong resistance toward additional mating attempts and frequently reject courting males. However, persistent males may occasionally achieve secondary mating after repeated courtship efforts. These observations indicate that multiple mating and mate choice play important roles in the reproductive biology of *Forcipomyia* sp.

Chemical communication further regulates mating interactions in these insects. Virgin females release volatile sex pheromones that attract males and stimulate courtship behavior. After mating, pheromone production decreases, enabling males to distinguish between virgin and mated females. This mechanism helps in reducing unnecessary mating attempts and improves reproductive efficiency within the population.

Although mating behavior has been studied in several dipteran insects, limited information is available regarding the combined effects of chronological age and multiple mating on the reproductive behavior of *Forcipomyia* sp. from the Rohilkhand Terai region. Therefore, the present study was undertaken to investigate the influence of age and repeated mating on female resistance behavior, copulation dynamics, and reproductive interactions in *Forcipomyia* sp. The study aims to provide baseline information on the reproductive biology of this species under natural and laboratory conditions.

OBJECTIVES

- This study investigated the combined effects of insect age, male body size, and female reproductive status (multiple mating) on the swarming, courtship, and chemical communication behavior of *Forcipomyia* sp.

MATERIALS AND METHODS

1. COLLECTION TECHNIQUES AND SOURCE OF MATERIAL

Adult Sampling: *Forcipomyia* adults were collected from natural habitats using mechanical aspirators. Swarming species were gathered via Diptera sweeping nets, and nocturnal individuals were sampled using overnight UV-light traps.

Pupae Extraction: Wild pupae were isolated from soil and sand using the Magnesium Sulphate ($MgSO_4$) flotation method, rinsed with distilled water, and sexed under a stereomicroscope based on pupal dimorphism.

Rearing and Emergence: Sexed pupae were placed in sealed Petri dishes with damp sand. Emergence was induced in an incubator at 30-35°C Emerged adults were transferred to 3x1 glass vials containing an inclined filter paper strip and a cotton wad soaked in 10% sucrose solution for nourishment.

2. TRANSPORT AND LABORATORY REARING

Field-collected pupae were stabilized within small sand samples to minimize mechanical stress and maintain moisture during transit to the laboratory. Once in the laboratory, insects were maintained under controlled environmental conditions 26± 2° C, 70--80% relative humidity, and a 12:12 hour light: dark photoperiod until they reached the specific chronological ages required for the experiments.

3. EXPERIMENTAL DESIGN FOR BEHAVIORAL ASSAYS

To evaluate the effects of chronological age and reproductive status, adults were grouped into specific age cohorts ranging from 1 to 10 days post-emergence.

Effect of Age on Virgin Receptivity: Virgin females of varying ages (1, 3, 5, 8, and 10 days old) were introduced individually to sexually mature virgin males. The time to achieve genital union, instances of pre-copulatory female resistance behavior (such as wing kicking or abdomen curling), and total copulation duration were recorded.

Effect of Male Age on Insemination: Virgin females were paired with males of distinct age cohorts (1–3 days, 4–8 days, and >8 days. The total duration of copulation was monitored to assess its relationship with sperm transfer efficiency

4. MULTIPLE MATING AND FEMALE RESISTANCE TRIALS

To investigate the impacts of reproductive status, individual virgin females were first allowed to mate with a high-fitness male. Following successful copulation, these now-mated females were exposed to subsequent courtship attempts by secondary males at different time intervals (2, 12, and 24 hours post-mating). The intensity of female resistance behaviors and the success rate of secondary inseminations were quantified and compared against the baseline behaviors of virgin controls.

5. DATA SUMMARIZATION AND BEHAVIORAL EVALUATION

All observations gathered from the laboratory pairing trials and field collections were directly compiled and organized based on their respective experimental groups. To track the impact of age and temperature on reproductive success, behavioral metrics-such as the total duration of copulation and the time required to achieve genital union-were summarized by calculating their clear arithmetic means (averages).

Similarly, qualitative actions, including the frequency of female pre-copulatory resistance behaviors (such as wing kicking and abdominal curling) and overall mating success rates, were converted directly into straightforward percentage values (%). These summarized percentages and averages were then systematically arranged into descriptive tables to look for clear behavioral trends and direct comparisons across the different insect cohorts without further mathematical transformations.

6. MULTIPLE MATING AND FEMALE RESISTANCE TRIALS

To see how a change in reproductive status reshapes behavior, individual virgin females were first paired up and allowed to fully mate with a high-fitness male. Once that initial copulation wrapped up successfully, these now-mated females were re-exposed to fresh advances from a second male at strict time intervals: either 2, 12, or 24 hours later. We tracked the intensity of their defensive rejections and checked whether these secondary matings were actually successful, comparing all numbers against the baseline behaviors of our unmated virgin controls.

RESULTS

1. IMPACT OF TEMPERATURE ON FECUNDITY AND HATCHABILITY

The reproductive performance and egg viability of *Forcipomyia* sp. were heavily influenced by temperature variations. The quantitative data tracking total egg production and subsequent larval emergence across five distinct temperature regimes 10°C to 40°C is summarized in Table 1.

Table 1: Effect of temperature regimes on *Forcipomyia* egg production and hatchability

S.No.	Temperature at °C	Total no of eggs laid by female	No. of hatched out duration of incubation	% hatchability
1.	10	218	125	57.3%
2.	18	248	158	63.7%
3.	22	265	186	70.2%
4.	30	205	174	85%
5.	40	789	48	25%

The total number of eggs laid by females increased progressively with temperature from 10°C 218 eggs up to a peak at 22°C 265 eggs, followed by a slight decline at 30°C 205 eggs. An extreme temperature-induced oviposition surge was recorded at 40°C yielding the highest count of 789 eggs.

However, egg hatchability followed a non-linear trajectory. The percentage of successful larval emergence rose consistently with temperature, climbing from 57.3% at 10°C to a maximum optimal hatch rate of 85% at 30°C Conversely, despite the high egg count observed at at 40°C environmental heat stress severely compromised embryo viability, resulting in the lowest recorded hatchability rate of just 25%,

2. IMPACT OF FEMALE AGE ON REPRODUCTIVE CAPABILITY AND FECUNDITY

Behavioral assays demonstrated that chronological age plays a critical role in modulating the reproductive capability and sexual receptivity of virgin *Forcipomyia* females. The fecundity of these females according to age is given in Table 2 below-

Table 2: Relationship between virgin female age, male mating attempts, and percentage receptivity

Female age	No. of females Attempted by the male10	No. of female shown receptivity	% Fecundity (Receptivity)
0-4 hrs	45	15	33%
5-15hrs	40	35	87.5%
16-25 hrs	40	30	75%
26-40hrs	40	15	37.5%
40-60hrs	40	5	12.5%
>60 hrs	unreceptive	-	-

The reproductive capability and fecundity of the female depend upon the age. Experiments show that the 15 out of 45 vergin female aged 0-4 hrs were complete receptive. Contarary to this 35 out of 40 virgin female age 5-15 hrs were found highly receptive. Female aged 16-25 hrs exhibit that 30-40 are receptive. Only 15 out of 40 females of aged 26-40 were found receptive. The minimum receptive was observed that is 5-10, in the female aged 40-60 hrs. Female aged more then 60 hrs were generally found unreceptive

3. STUDY ON THE SEX RATIO AND ITS SIGNIFICANCE

To determine the sex ratio, samples of imagoes were taken from the field and the result are given here under (Table 3)

Table 3: Quantitative field analysis of swarm density and sex ratios in *Forcipomyia* species

Density range of individuals per 5 sweeps of net	No. of male	No. of female	Sex ratio	Average
F. dibiyapurensis				
421	410-415	4	104/1	106/1
502-530	490-522	5	103/1	
349-368	320-344	3	106/1	
496-512	460-480	5	102/1	
220-222	190-212	2	110/1	
F. barkhai, F. mananthrai, F. dasguptai				
4200	4110-4140	35	107/1	105/1
1675	1605-1650	15	111/1	
1421	1390-1408	14	101/1	
1704	1684-1690	16	106/1	
5600	5502-5542	55	101/1	

A careful study of the research, reveal that density of individual in swarm varies from 220-530 per five sweeps of the net in the *F. dibiyapurensis*, where as in *F. barkhai*, *F. etawahensis*, *F. dasguptai*, *F. mananthrai*, it ranges between 1390-5542 individual only, thus indicating a numerical superiority of the former over the latter. In the sex-ratio, also, there is a marked difference between male/female ratio varing from 102-110 as compared to another where it varies from 101-111.

It can be concluded that these swarm plays an important role in bringing to sexes together for mating. As apperent from the above discussion that the female ratio in comparison to male is very low i.e. 101:1 to 111:1. Therefore, there are 101-111 male per female. Although the number of females very low in the swarm sample, the male still exhibits multiple mating behaviour

4. BEHAVIORAL PARADOX AND EVOLUTIONARY ADAPTATION (ALTRUISM)

Despite the extremely low female representation inside the swarms, males continued to exhibit multiple mating behaviors. This highly paradoxical reproductive situation-where thousands of males compete intensely for a minimal number of females-can be interpreted through the evolutionary framework of altruism (specifically, *unadone altruism*).

Under this hypothesis, unadone altruistic mechanisms restrict long-term individual reproductivity for certain males through their energetic participation in swarming assistance, while the successful recipient male gains heightened offspring survivability. This phenomenon represents a specialized reproductive adaptation within biting midges. When a helper or donor directs its swarming and acoustic support toward close genetic relatives, the reduction in its independent reproductive success is evolutionary compensated by increasing the overall inclusive fitness and reproductive output of its related kin line. This points to a potential system of male co-operation within *Forcipomyia* swarms to ensure population survival, though this hypothesis requires further experimental confirmation.

DISCUSSION

1. THERMAL STRESS AND OVIPOSITION RESPONSES

The high surge in egg production at 40°C (789 eggs) likely represents a terminal stress response where females release all remaining eggs under lethal conditions. However, the drop in hatchability to 25% demonstrates that high heat compromises embryo viability. Optimal conditions are constrained around 30°C, achieving the highest hatch efficiency (85%).

2. AGE-DEPENDENT MATING DRIVE

Newly emerged females (0–4 hours) exhibit low mating drive due to ongoing physiological development. Receptivity peaks sharply within the 5–15 hours window (87.5%). Beyond 24 hours, reproductive senescence causes a steep drop in mating willingness, progressing to complete non-receptivity after 60 hours.

3. POST-COPULATORY REGULATION AND SPERM TRANSFER

- **Sperm Transfer**: During copulation, the male transfers sperm efficiently.
- **Gland Secretions**: Accessory gland secretions are simultaneously transferred (Klowden 1999).
- **Inhibiting Remating**: These biochemical secretions actively inhibit female remating.
- **Inducing Oviposition**: The chemical signals successfully induce rapid oviposition.
- **Female Exit**: After copulation, females rapidly leave the swarm (Yuval *et al*. 1993).
- **Male Persistence**: Conversely, males stay inside the swarm to conserve localized positions.
- **Secondary Seeking**: Males continuously seek another virgin mate.

4. THE ALTRUISM PARADOX AND MALE COOPERATION

The highly skewed male-biased sex ratio (101:1 to 111:1) within swarms presents a distinct operational paradox. This situation can be explained by the phenomena of altruism (unadone altruism). Unadone altruism is a term restricted to cases in which the individual really does lose reproductivity over the long haul as a result of its help, while the recipient gains more survivability of offspring.

This phenomenon can be considered a beneficial reproductive adaptation in the species of biting midges. In the above observations, it is clear that if a helper or donor directs its aid to its genetic relative, it may be more than compensative for a reduction in its own reproductive success by increasing the reproductive success of the related individual. The swarm sample analysis also indicates that there may be a possibility of cooperation among males so that their race can survive for the future.

CONCLUSION

Mating success in *Forcipomyia* sp. is strictly governed by environmental temperature, chronological age, and physical constraints. The extreme male-biased sex ratio drives severe pre-copulatory competition, forcing the development of male cooperative swarming strategies via unadone altruistic mechanisms. To ensure effective field suppression, mass-reared male midges must be maintained within optimal thermal conditions and released at peak reproductive age thresholds.

SIZE AND AGE DETERMINANTS:

Male body size (wing length) is a critical factor in wild population dynamics; males caught in copula within swarms were significantly larger than non-mating individuals. The optimal age for male insemination success was 4–8 days. However, increasing male age significantly prolonged the duration of copulation, indicating slower sperm transfer.

FEMALE RECEPTIVITY AND AGE:

As virgin females aged, their pre-copulatory resistance behavior significantly decreased, resulting in a shorter time required to achieve genital union.

MULTIPLE MATING AND RESISTANCE:

Previously mated females exhibited intense resistance toward subsequent male mating attempts. Secondary matings were rare and only achieved through the persistent efforts of highly fit males, serving as a mechanism for female mate choice.

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