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Flying Sperm: The Indispensable Component of the Instrumental Insemination of Honey Bees: A Review

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ABSTRACT

Honey bees are significant pollinator species in both natural and agroecosystems. Among three honey bee castes, drones are often regarded as "lazy willi" and assumed merely to function as "flying sperm" in the honey bee colonies, this view is incorrect. During take-off and landing, they always exhibit higher thermogenic capacity than workers, in addition to colony thermoregulation, they received higher attention for astonishing mating behavior. Honey bee queens are highly polyandrous and mate in mid-air with many drones from diverse genetic sources. Instrumental insemination is an essential tool that provides complete control of honey bee mating for breeding and research purposes. Controlled mating in honey bees helps to maintain economically valued traits that ensure colony productivity and sustainability. Ultimately, breeding and keeping better bees through instrumental insemination offer improved pollination that guarantees global food safety and security. The present review emphasizes mainly on the physiology and mating behavior of drones in the context of instrumental insemination.

Key words: Breeding, Drone, Instrumental insemination, Thermoregulation.

Honey bees play a crucial role in the pollination of 80 per cent of crops and also contribute to the production of 1.6 million tons of Life's sweetener (Honey) (FAO, 2015). The estimated losses due to inadequate pollination in India are around Rs. 10,000 to Rs. 55.000 per hectare for cross-pollinated crops (Mohapatra *et al.,* 2010). Honey bees possess haplodiploid sex determination in which unfertilized (haploid) embryos develop into males while fertilized (diploid) eggs develop into females. Extensive studies were carried out on queens and workers among different bee castes of the colony and overlooked the biology and behavior of drones (Reyes *et al.,* 2019). This is not shocking as drones are not directly used for commercial purposes; besides they are only raised and present in honey bee colonies for short durations (Free and Williams, 1975) and eliminated from colonies when winter approaches. Even though drones are not engaged in food collection or brood care, the heat produced by drones is known to contribute to the nest's thermoregulation (Harrison, 1987; Kovac *et al.,* 2009). Drones have mostly gained attention because of their extraordinary mating behavior outside the nest, which leads to rapid outbreeding in honey bee populations (Baudry *et al.,* 1998). Recent concerns about the declining numbers and health of honey bees highlight the importance of studying their drone congregation areas (DCAs) (Giray *et al.,* 2010; Mullin *et al.,* 2010). Genomic diversity and genomic structure can be estimated more easily when DCAs are identified (Collet *et al.,* 2009).

The honey bee colonies produce competitive drones that mate either with queens from other colonies or queens of the same colony, passing on their genetic material to the following generation. In social insects, natural variation and selection are very important to sustain superior qualities. A "major driver of natural selection in honey bees" is male reproductive success and "selection through the male side appears to be an extremely important factor for colony fitness" (Bejan and Kraus, 2003). Controlled mating will pave the way for producing desired traits in the offspring of any organism. In honey bees, controlled mating can be achieved by the special technique called Instrumental insemination in which, semen from the drone of interest will be used to inseminate the desired queen. As the female characters are fixed for a particular honey bee colony, male characters can be chosen through controlled mating. Hence, drones play a vital role in sustaining the desired traits in the honey bee colonies and should be given proper importance.

Drone bee physiology

The physiology of honey bee drones has not been studied as thoroughly as that of workers and queens. Drones have

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evolved anatomically and physiologically to enable powerful and vicious flight (Radloff *et al.,* 2003) and successful semen transfer through their intricate reproductive organs (Koeniger and Koeniger, 1991). Additionally, wax glands, hypopharyngeal glands (*i.e.* brood-feeding glands) and the majority of food-collecting structures are absent in drones.

Development and nutrition of drone larvae

Honey bees lay fertilized and unfertilized eggs, which may differ clearly or slightly (Henderson, 1992). The food given to young drone larvae is milky white in appearance and changed to a dirty yellow-brown color later. The color change is mainly due to the addition of pollen and honey leading to reduced protein and fat contentand increased carbohydrate content in the old-age drone larvae than young drones (Haydak, 1970) which leads to delayed drone development compared to workers (Stabe, 1930). Unfertilized eggs required 3.6±1.0 h longer to develop than fertilized eggs (Harbo and Bolten, 1981). Drone larvae weighed 262-419 mg (Hrassnigg and Crailsheim, 2005).

To produce drones, the queen lays unfertilized eggs in larger comb cells called ‘drone cells’, when her sperm storage is depleted for various causes, some unfertilized eggs may be laid in smaller worker cells. Rearing these eggs produces smaller drones compared to those produced by drone cells (Berg, 1991). In Apoidea, different male morphs are associated with distinct behaviors (Berg *et al.,* 1997). Small-sized drones showed lower spermatozoa (7.5±0.5 million) but 20 per cent more spermatozoa were observed in normal-sized drones (11.9±1.0 million) (Schluns *et al.,* 2003). Smaller drones have reduced reproductive success (Berg *et al.,* 1997). Drone brood cells were preferred by parasitic mites, *Varroa jacobsoni* and its invasion drastically reduced the weight of a newly emerged drone from 277-290 mg to 250.4 mg (Duay *et al.,* 2003; Hrassnigg and Crailsheim, 2005).

Drone larvae gained 2-3 times their body weight in the first several days after hatching and 10-fold during the fourth and seventh day of their existence (Lipinski *et al.,* 2008). Carbohydrate concentrations fluctuated throughout the drone brood, from newly born larvae to imago. The glycogen concentration ranged from 127.4 to 6.1 mg/g in pink-eyed pupae and freshly emerged drones, respectively (Szolderits and Crailsheim, 1993). Similarly, other carbohydrates of trehalose concentration varied from four-day-old larvae (1.5 mg/g) to pupae (12.9 mg/g) and glucose concentration ranged from 1.3 to 3.0 mg/g fresh matter in pupae. (Hrassnigg and Crailsheim, 2005; Schmolz *et al.,* 2005).

Physiology of digestion in drone bees

Drones ingest pollen only for the first few days after adult emergence, increased proteinaceous intake reduces the drone’s appetite, resulting in lower pollen intake (Szolderits and Crailsheim, 1993). Drones consume less pollen than workers because they do not make royal jelly to feed developing larvae. Drones flying out of the colony had an average weight of 16.1 to 30.0 mg, while, returning drones had an average weight of 2.5 mg (Free, 1957). Even though drones appear to have decreased honey stomach capacity compared to workers (Snodgrass, 1910) they provide enough energy for mating flights, implying that they are not involved in foraging. Drone’s low crop content and limited glycogen reserves pose a considerable danger of starvation during tracking flights (Hrassnigg and Crailsheim, 2005).

The additional purpose of the hypopharyngeal glands in workers is to the generation of different enzymes like α-glucosidases, β-glucosidase, glucose oxidaseand amylase to digest carbohydrates and pollen (Ohashi *et al.,* 1999). Since drones lack hypopharyngeal glands, certainly, these glands do not produce these enzymes in drones (Snodgrass, 1910). There are no discernible differences between workers and drones regarding midgut enzymes like lipase, proteases, sacrase, maltase, lactase, *etc*. (Pavlovsky and Zarin, 1922). However, there are distinct qualitative variations within the protease class (Giebel *et al.,* 1971). Drones had less proteolytic activity than workers as the pollen intake rate is comparatively less in drones, workers feed the young one and the drone with protein-rich secretions in addition to carbohydrates (Crailsheim, 1991). The amylase activity of a drone’s midgut does not digest starch quickly for flight, but workers can use fed starch as fuel (Hrassnigg *et al.,* 2003). This information highlights how workers’ primary role is to break down food for the colony and demonstrates how drones depend on this pre-processed food.

Energy metabolism of drone bees

Drones rely heavily on carbohydrate meals to fuel their flight muscles. The two days older drone generates heat, particularly at low ambient temperatures. Research on temperature-related energy metabolism in drones is limited compared to those of workers. Caged drones with varying ambient temperatures demonstrated distinct behaviors. Drones were ectothermic at temperatures ranging from 5 to 20°C, but they were endothermic in temperatures ranging from 25 to 35°C and the chill coma temperature at which an animal goes motionless, is higher in drones (~14°C) than in workers (~11°C) (Free and Spencer Booth, 1960). The extinction temperature of flight muscles is the point at which no more muscle potential amplitudes are formed and flight muscles cannot be engaged (Goller and Esch, 1990). The drone’s extinction temperature (13.3±1.2°C) was 1-2°C higher than that of the workers (11.2±0.7°C).

Drones are more affected by low temperatures (<20°C) (Goller and Esch, 1991), so they typically depart the colony at warmer temperatures (>20°C) for longer flights. Drones must maintain thermal stability during flight by cooling or adjusting their metabolic rate, in addition to reaching a particular temperature (Harrison and Fewell, 2002; Moffatt, 2001). Drone’s flight muscles are not completely formed when they first emerge. Foraging workers’ muscles have a high concentration of respiratory enzymes per unit mass, which are supported by numerous mitochondria and cristae (Suarez *et al.,* 1999; Suarez *et al.,* 2000). Malate dehydrogenase activity will reach its maximum level in drones earlier than in other castes (Moritz, 1988). There is no information on pyruvate kinase or citrate synthetase in drones (Harrison, 1987). Drones may follow a tethered queen at a speed of 5 m/s and they may have an advantage in aggressive encounters between males due to their larger body mass and increased physical energy (Coelho, 1996; Koeniger, 1988).

Thermoregulation by drone bees

In their close-knit communities, honey bees rely on one another for survival and procreation, with each member exhibiting a highly integrated behavioral pattern. Honey bees are insects widely recognized for their unique capacity for social thermoregulation. They maintain the temperature of brood nest between 32-36°C (Jones *et al.,* 2005). There are frequently unwelcomed outcomes if nest temperatures are not maintained within the ranges particular by each species (Jones and Oldroyd, 2006). Because honey bee broods are highly stenothermic, precise temperature control is essential in ensuring their optimal growth and development (Groh *et al.,* 2004). For a while, eggs and larvae in open brood cells may withstand lower temperatures; however, pupae in sealed brood cells are extremely sensitive to cooling (Groh *et al.,* 2004; Tautz *et al.,* 2003). There was a high incidence of shriveled wings and legs, deformities of the abdomen and in severe cases, behavioral and neurological deficits were observed in emerging adults when they spent prolonged amounts of time below 32°C (Tautz *et al.,* 2003).

Heat production of honey bees varies in worker bees, drones and queen bees. The adult single worker exhibited the highest rates of heat production of 209 mW/g and the mean heat generation rate exhibited by juvenile workers was 142 mW/g. There are heat variations observed in egg-laying (102 mW/g) and virgin (117 mW/g) queens. Drones exhibit significant variations in their heat production rates. Due to their greater locomotor activity, juvenile drones produced 68 mW/g of heat, whereas adult drones produced 184 mW/g (Kovac *et al.,* 2009). Nevertheless, it is indisputable that drones may generate heat because, during flight, they raise their thoracic temperature to 39.6-43.1°C, which is more than in workers (Kovac *et al.,* 2009). Drones require a greater thoracic temperature during pre-flight and warm-up to achieve lift-off. Drones that depart and return to the hive had a greater mean thorax temperature compared to workers (Coelho, 1991). Because of the larger size each drone will contribute 1.5 times much heat than worker to colony thermogenesis (Harrison, 1987).

Drone congregation area and mating behavior

In many Apoidea, mate locations fluctuate, mating takes place at feeding areas, emergence sites, oviposition sites, flowers, or locations that are exclusively or mostly attended for mating (Eickwort and Ginsberg, 1980). In the genus *Apis* copulation appears to occur high in the atmosphere, an evolutionarily viable mating arrangement that serves against inbreeding (Page Jr, 1980). *Apis mellifera* L. drones congregate high in the air at various sites every day during the mating season and every year, regardless of the presence of queens and these places are known as “Drone Congregation Areas” (DCAs), which play a crucial role in studying bee behavior and can be used for genetically controlled mating in breeding programs and defining conservation zones for honey bee subspecies (Ruttner, 1976). The reasons that attract drones and queens to DCAs are hypothesized as the “physical DCA hypothesis” (Galindo-Cardona *et al.,* 2012), while it is well-established that notable topographical features are crucial for drone orientation (Loper *et al.,* 1992). One alternate hypothesis is the “behavioral DCA hypothesis” that suggests the behavioral interactions between flying drones and queens could lead to DCAs (Loper *et al.,* 1992). Drones undergo an 8-to-10-day period of sexual development before beginning to fly to drone congregation places and in *A. mellifera* five to seven days old queens fly to DCA (Colonello and Hartfelder, 2003). A queen copulates with ten to twenty males during the mating flight and after a few days, she begins to lay eggs (Estoup *et al.,* 1994). *Apis* queen’s sex attractant mainly comprises 9-oxo-trans-2-decenoic acid to which all the *Apis* drones are attracted, but one of the main causes of interspecific reproductive isolation is assumed to be the variation in the mating flight time of sympatric *Apis* species (Koeniger, 1988). Conductive results on DCAs cannot be entirely drawn because it is extremely challenging to guarantee the same situation in the laboratory for the studies (Paxton, 2005).

To the genus *Apis*, aerial copulation has to be regarded as “autapomorph”. The actual purpose of a drone is to produce sperm and to mate with a queen, even after the drone has died, the mated queen retains the viable sperm in the spermatheca for the duration of her existence (Klenk *et al.,* 2004; Phiancharoen *et al.,* 2004). To mate, a drone must locate an aerial queen at a drone congregation area and then engage in competition with numerous additional drones (Berg *et al.,* 1997). The *Apis* genus exhibits significant levels of polyandry and multiple copulations (Rinderer *et al.,* 1998). Multiple mating is preferred for genetic variation, caste differentiation; and a variety of genotypes. Queens never mate again after they begin laying eggs, even if they run out of sperm (Winston, 1991) (Fig 1).

The competitive behavior of males fetches larger drones to congregate in areas where queens are easily available (Alcock and Houston, 1987). Reasons for the reproductive disadvantage of smaller drones are their low reproductive capacity, low amount of sperm, small size of spermatophore and small copulation duration (Thornhill and Alcock, 1983). No differences in the number of spermatozoa between the two drone kinds in *A. mellifera* (Berg, 1991). The duration of copulation in honey bees is determined by the drone’s loss of motility during endophallus everting, which occurs at the beginning of the process in both small and normal-sized drones. Reduced

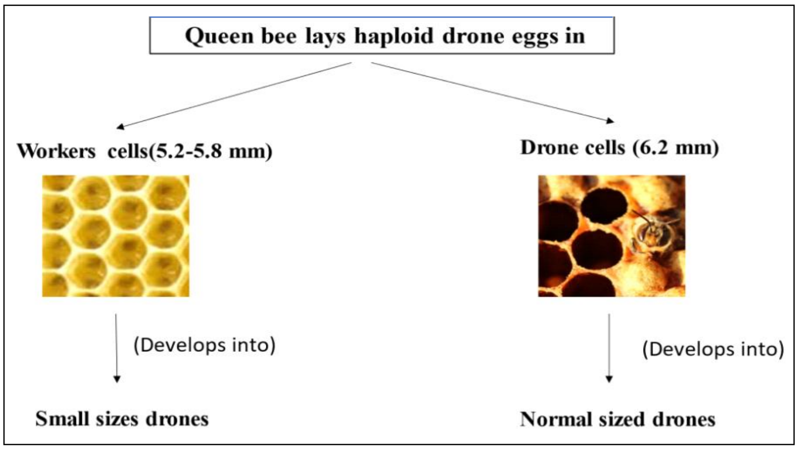

Fig 1: Honey bee queen, laying haploid eggs in two different types of cells (Schluns *et al.,* 2003).

individual inefficiency during the copulatory process is not the primary cause of smaller drones' decreased reproductive success; rather, it stems from a lower success rate in the competition for access to the queen at the drone congregation area (Berg *et al.,* 1997). Though small drones produce 20 per cent more spermatozoa because of their lower flight, capacity colony prefers to rear normal-sized drones rather than small ones (Schluns *et al.,* 2003).

Mating behavior of *Apis mellifera*

In *Apis mellifera*, mating never takes place inside the colony rather, it happens at flyways or "drone congregation areas" (Koeniger *et al.,* 2005). At DCAs, hundreds of males and fertile females ("queens") gather, particularly in the afternoon (Galindo-Cardona *et al.,* 2012) for mating, which occurs at heights of 15-60 meters, with flying bees reaching speeds of up to 12 km/h (Loper *et al.,* 1992) (Fig 2). *Apis mellifera* queen mating flight transpired between 13.18 and 14.48 h. The mean flight time of *A. mellifera* drones is between 13.56 and 16.26 h (Yoshida *et al.,* 1994), producing approximately 10 to 12 million spermatozoa (Woyke, 1975).

In places with extensive beekeeping, about 12 to 15 thousand drones visit drone congregation areas (Koeniger *et al.,* 2005). Mating flights often last for 10 to 30 minutes (Ruttner, 1976). More than one mating flight was experienced by 27 to 60 per cent of queens (Tarpy and Page, 2000). Whereas 10 per cent of the queens fly away more frequently (Schluns *et al.,* 2005; Woyke, 1964). Throughout various insect species, males obstruct rivals during mating by using secretions from accessory glands as mating plugs (Thornhill and Alcock, 1983), in the contrary, the *A. mellifera* drone uses its secretions to identify the queen and aid in further copulation by making it easier for other drones to recognize her (Koeniger, 1990). This tendency, called "post-mortem cooperation" also ensures that the number of matings within a mating flight period is minimized (Koeniger and Koeniger, 2007). The number of copulations and spermatozoa in the spermatheca is related to the threshold for successful matingand on the mating flight, queens gain information regarding the effectiveness of mating (Koeniger and Koeniger, 2007).

Mating behavior of *Apis cerana*

Apis cerana F. has four subspecies, *A. cerana cerana*, *A. cerana japonica, A. cerana indica, A. cerana himalayana* (Ruttner, 1988). The *A. cerana indica* queen mating flight is known to occur between 13.26 to 14.26 h in Japan (Woyke, 1975), 12.40 to 13.40 h in India (Verma, 1991), 13.15 to 16.15 h in Pakistan (Ruttner *et al.,* 1972) and in Sri Lanka, it occurred between 16.07 to 16.47 h (Punchihewa *et al.,* 1990). *A. cerana indica* drone's mating flight was documented between 13.29 to 15.14 h in Borneo (Koeniger, 1988), 15.22 to 16.52 h (Punchihewa *et al.,* 1990) and between 16.07 to 17.07h (Koeniger and Wijayagunasekera, 1976) in Sri Lanka, 11.15 and 15.15 h in Pakisthan (Ruttner *et al.,* 1972)and 13.25 and 14.55 h in India (Verma, 1991). In *A. cerana japonica* mating flights occurred between 15.03 to 16.18 h in queens and 13.33 to 16.48 h in drones and both required more time than those of *A. cerana indica* (Yoshida *et al.,* 1994).

Drone comb

Using drone combs and increasing drones in the colony always circles ambiguities. The proliferation of drones ought to be discouraged, by taking out the drone comb and replacing it with worker cells (Seeley, 2002). Finding just how much a beekeeper gains from taking drone comb out of his hives would be beneficial, particularly in the non-pesticide treatment of the troublesome mite Varroa destructor (Sammataro and Avitabile, 1998). A small number of investigations concluded that, adding a drone comb to a colony and thereby expanding its drone population does not lessen its capacity to make honey (Johansson and Johansson, 1971). Conversely, other experiments revealed that there was a noticeable difference in the weight growth (honey production) between the drone comb-equipped (25.2 kg) and drone-free colonies (48.8 kg). It is concluded that colonies with a natural drone comb produce less honey than colonies with minimal or no drone comb (Seeley, 2002). The results were justified as drone rearing encourages swarming and drone rearing and maintenance is expensive and encourages Varroa reproduction (Sammataro and Avitabile, 1998).

Instrumental insemination in honey bees

Honey bees pose a special challenge of controlled mating to regulate, because of their natural mating behavior. Queens are highly polyandrous (Gencer *et al.,* 2014) and typically mate with 30-70 drones (yaniz *et al.,* 2020) while in flight, in places where 10,000-30,000 drones from various genetic origins congregate (Koeniger, 1986). Instrumental insemination is a vital tool for research and breeding purposes, which offers total control over honey bee mating. Instrumental insemination of *A. mellifera* queens began in the 1920s (Cobey, 2007). The innovative instrumental insemination technology has several applications which include, a single drone can inseminate one or even multiple queens (Van Praagh *et al.,* 2014), to produce colonies with desirable traits (like pollen hoarding, Varroa mite resistance, hygienic behaviors) (Huang *et al.,* 2009; Khan *et al.,* 2021) and honey bees are made to mate in a controlled manner to produce crosses that occur beyond the nature (Cobey *et al.,* 2013).

Insemination technique

i)Eversion of the honey bee drones for insemination into the queen

Semen can be collected in two different ways, one directly from the seminal vesicle of the droneand another one by induced ejaculation (Yaniz *et al.,* 2020) which involves exposing the semen to induce ejaculation. Endophallus is everted by hand in two steps; Partial eversion and complete eversion.

ii)Semen collection

Semen from the drone's everted endophallus is collected into a glass capillary tube connected to the syringe (Yaniz *et al.,* 2020). Semen from the drone is collected directly into a syringe easily with the saline recipe. During semen collection avoid collecting the viscous mucus layer which clogs the syringe tip and air bubbles in the syringe. Drones are more likely to defecate during the eversion and discard them.

iii)Insemination of the queen

The virgin queen of five to seven days of post-emergence is given carbon dioxide treatment twice, one before the day of insemination and second during the procedure of insemination for one to four minutes. Carbon dioxide administration anesthetizes the queen during the procedure and is proven to stimulate oviposition.

iv)Field dissection of honey bee queen spermatheca

Sperm migration from the median oviduct to spermatheca in the inseminated queen takes around 40 hours. Spermatheca is white with a spherical structure about one millimeter in diameterand the surface with trachea net covering gives a rough surface texture. Spermatheca varies, clear in the virgin queen, tan with a pattern of marbled swirls in the mated queenand white in the poorly mated queen (Cobey *et al.,* 2013).

Instrumental Insemination of *A. cerana*

A. cerana produces an average of 0.09-0.1 µl of semen per drone, to extract 1 µl of semen, 11.94 drones must successfully discharge their semenand 17 drones must be killed. Unlike naturally mated queens, artificially inseminated queens that used 4 µl of semen (once insemination) or 8 µl of semen (twice insemination, each with 4 µl of semen) began depositing eggs 2.5 days later. One time instrumentally inseminated queen with 4 µl semen was favorable and started laying as a naturally mated queen (Vung *et al.,* 2016).

Instrumental Insemination of *A. cerana indica*

A cerana indica drones produce 0.20 µl semen, with a concentration of 4655 thousand spermatozoa per microliter,

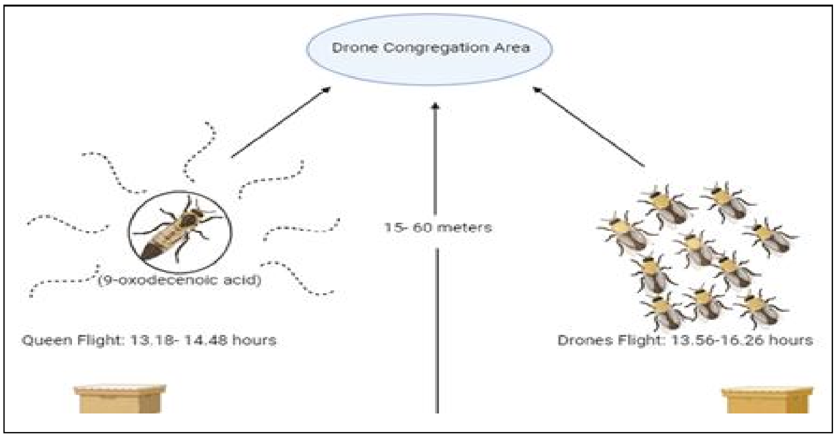

Fig 2: *Apis mellifera* queen and drones moving towards drone congregation area.

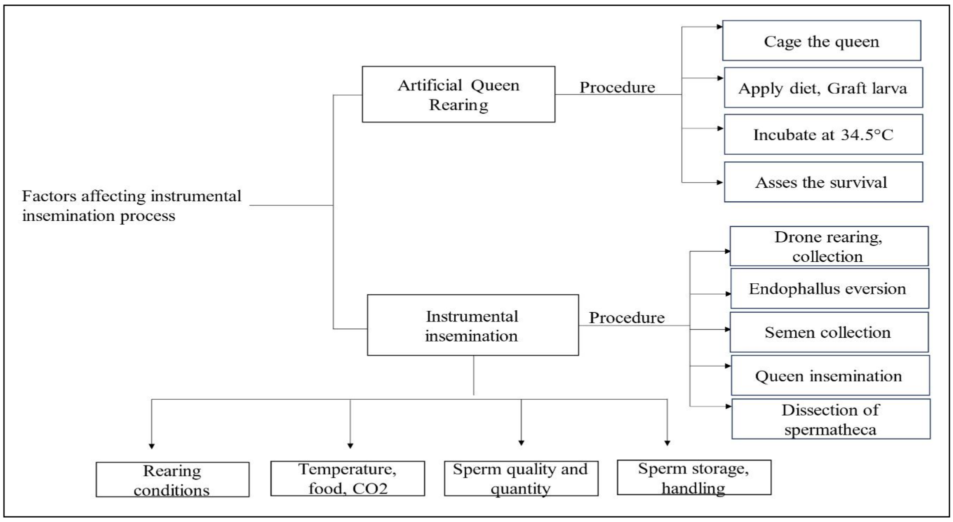

Fig 3: Factors affecting instrumental insemination process (Khan *et al.,* 2022).

the mean number of spermatozoa produced per drone was 1000-1500 thousand (Ruttner *et al.,* 1972). The number of spermatozoa in the spermatheca was considerably enhanced by increasing the volume of injected semen from 1 to 4 µl in the instrumentally inseminated queen when temperature increased from 28-34°C. Unlike queens that were inseminated twice with a smaller amount, some queens that were inseminated once with a significant dose of semen did not have their oviducts emptied. Queens inseminated with higher than 3 µl semen were producing worker brood in the season (Woyke, 1973). In the 6 µl of semen taken from 20-36 drones, the *A. cerana indica* queen was instrumentally inseminated twice. The spermatheca contains an average of 1.4 million spermatozoaand the queen can lay viable eggs for a year (Woyke, 1975).

Instrumental insemination

Assessing *A. cerana indica* and *A. mellifera*

Reproductive structures such as the testes and ovaries of *A. cerana indica* were smaller than those of *A. mellifera*, but the other parts were similar in size (Kapil, 1962). Compared to *A. cerana indica* drones, *A. mellifera* drones produce 7.5 times more semen (Woyke, 1975) and 11 times more spermatozoa. *A. cerana indica* spermatozoa had a lower efficiency of entry to the spermatheca (5·4 to 7·6 per cent) compared to *A. mellifera* (17·3 to I2·4 per cent), likely due to lower concentration and penetration of the semen and smaller spermatheca size (Mackensen, 1964). The average concentration of spermatozoa in *A. mellifera* (after insemination with 1 mm^{3}semen) was 1428 thousand per mm^3, which was approximately double that of *A. cerana indica* queens inseminated with the same volume (752 thousand per mm^3) (Woyke, 1971). *A. mellifera* queens require approximately 8 drones for successful insemination, while *A. cerana indica* might need 40-60 drones. In the process of insemination to inseminate the queens, 6-24 drones must be crushed in *A. mellifera* and 100-150 in *A. cerana indica* (Woyke, 1973). Compared to *A. mellifera*, the semen of *A. cerana* is far more difficult to separate from mucus. Despite being smaller than *A. mellifera queens*, *A. cerana indica* queens have relatively easy instrumental insemination because it is simpler to inject semen into their oviducts in this species (Woyke, 1973).

Current status of instrumental insemination technique

Instrumental insemination is a useful technique for more accurate breeding value estimation and reproductive control in genetic selection. Nevertheless, this method does not produce queens of exceptional genetic quality for commercial use (Maucourt *et al.,* 2023). Drone semen collected for instrumental insemination is analyzed to detect the presence of viral genome and the genomes of five viruses, namely deformed wing virus (DWV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV), sac brood virus (SBV) and *A. mellifera* filamentous virus (AmFV) were found in honey bees, focused on the venereal transmission of important honey bee pathogens (Prodelalova *et al.,* 2019). Many factors affect the performance of instrumental insemination such as rearing conditions, stress, inseminator's skills, food availability, mating age of queen, queen banking, temperature, sperms stored in the spermatheca, sperms quality and quantity, semen handling and storage, carbon dioxide and nitrogen treatmentsand pheromone. The productivity of the colonies headed by the instrumentally inseminated queens is higher in comparison with the naturally mated queens if all the affecting factors are optimized (Khan *et al.,* 2022) (Fig 3).

CONCLUSION

The increasing challenges of pests, parasites, pathogens and colony collapse disorder in honey bees warrant an urgency to find sustainable solutions for rescuing their population from dwindling. Selective breeding, stock improvementand preserving and maintaining the genetic diversity of honey bee ecotypes and subspecies are prerequisites to ensuring honey production and sustaining crop yield enhancement for global food security. As discussed in this review paper, the instrumental insemination technique allows us to achieve the goal of protecting honey bee genomes alive, warding off all demerits that challenge them in the modern era.

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Author contributions

V.R. Saminathan and M.R. Srinivasan conceptualized the experiment, K. Shweta collected reference articles and drafting of original manuscript, C. Sowmiya, GP, M.R. Srinivasan, VS and N. Manivannan refined the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

There are no relevant financial or non-financial competing interests to report.

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