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REVIEW

Drone Fertility: The Secret of Honey Bee Sustainability

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Abstract

Honey bees play a crucial role as pollinators in balancing the elements of the floral and faunal ecosystems. In honey bee colonies, male bees (drones) are crucial for population sustainability, offspring production, and the maintenance of phenotypic and genotypic variation. However, male bees have often been overlooked in apicultural research. The viability and potency of drone sperm production are critical factors influencing the health of queen bees and their subsequent progeny. In conclusion, factors that affect drone viability will also affect the progeny within colonies. This study highlights several key characteristics, such as semen volume, sperm concentration, viability, transfer dynamics, and testicular morphology, to assess drone fertility, which would provide basic facts in assessing sperm potency and ability to fertilize the female while undergoing newer breeding methods in bees. An overview of the genes responsible for spermatogenesis in drone bees, along with the roles of metabolic constituents like glandular proteins, is necessary to understand genetic diversity in bee populations. This review underscores the necessity of drone fertility as a key determinant of colony cohesion, evolutionary fitness, and adaptive capacity of honey bee populations in changing environmental contexts.

1. Introduction

Honey bees (*Apis spp.*) are essential pollinators for numerous agricultural and horticultural crops globally (Ghosh et al., 2020). Honey bees play a key role in pollination through refined communication mechanisms such as the waggle dance (Riley et al., 2005). Their pollination services are crucial, contributing to one-third of the human food supply. In the agricultural sector, honey bees are a cornerstone of sustainable production, as they play a pivotal role in pollination (Gallai et al., 2009). The predominant species include *Apis dorsata* F. (Rock bee), *Apis mellifera* L. (Italian bee), *Apis cerana indica* F. (Indian bee), and *Apis florea* F. (Dwarf Honey bee), with *A. mellifera* and *A. cerana indica* being extensively managed in commercial apiculture (Crane E, 2009). Hypopharyngeal gland development in worker bees plays a critical role in brood nourishment and colony functioning (Ahmad et al., 2021), while the drone population significantly enhances pollination efficiency by stabilizing the population build-up and sustaining genetic diversity within the colony. Genetic variability, predominantly influenced by the drone population, is critical for producing viable offspring. The drone and worker populations are subject to numerous biotic and abiotic factors within the ecosystem. Queen polyandry promotes mating with multiple drones, thereby increasing genetic diversity within colonies and improving colony fitness (Tarpy D.R, 2003). Drone Congregation Areas (DCAs) are defined spaces where drones aggregate to mate with queen bees exhibiting

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a marked tendency toward site fidelity and repeated use of specific locations. Colony density is quantifiable by successful mating events that result in viable progeny (Hagan T, 2024). Environmental stressors, particularly thermal fluctuations, can negatively affect drone reproductive performance by altering semen quality and reducing the number of spermatozoa transferred to the queen's spermatheca (Bieńkowska et al., 2011). The integrity of genetic material, particularly sperm quality, is vital for queen bee longevity (Pettis et al., 2016). Furthermore, locally adapted bee populations might provide insights into genomic vitality and robustness (Dogantzis et al., 2018). In this context, our study aims to analyse the significance of drone involvement in colony synergy, the physiological aspects of seminal fluid, drone reproductive capabilities, and the various metabolic and physiological pathways that underpin colony sustainability.

2. Biology of Drones in Honey Bee Colonies

In honey bee colonies, worker bees and queens arise from fertilized diploid eggs, whereas drones develop from unfertilized haploid eggs. The developmental timeline for drone bees typically spans 24 to 28 days, though it can vary with genetic and environmental factors (Brutscher et al., 2019). Factors such as brood nest temperature, worker population density, and available food resources can significantly influence the behavior and dynamics of the drone population. It is proposed that worker density in the brood area plays a pivotal role in the deposition of haploid eggs, while the emergence of male pupae initiates growth of queen larvae (Schultner et al., 2017). Upon eclosion, drones transport sperm to their accessory testes, remaining sexually immature until they reach sexual maturity within a timeframe of six to twenty days (Collins A.M, 2004). Sperm is typically stored in the seminal vesicle before it transits to the testes during eclosion (Lago et al., 2020). Drones begin migrating to seek mating opportunities with queens only after reaching sexual maturity, which occurs approximately 7 to 8 days after eclosion. The fertilization efficiency of the queen depends upon optimal migration of the seminal fluid during mating. In the designated Drone Congregation Areas (DCAs), multiple drones compete for queen mating. Mating occurs aerially in the DCA area, where large numbers of sexually mature drones congregate (Reyes et al., 2019). In the mating process, only the selected drone successfully inserts its endophallus into the oviduct of the queen while other drones surround them, forming a defensive "comet" structure. The eversion of the endophallus is eversible by mucus and lipid secretions from the cornual gland located near the cervical plates. This complex interaction concludes in a robust bond between the drone and queen, strengthened by sticky orange secretions. The brief copulatory process propels sperm and seminal fluid into the oviduct. The drone becomes dead after mating due to the detachment of the endophallus during the post-mating process, a phenomenon termed "suicidal mating" (Koeniger et al., 2005).

To optimize fertilization, queen bees contract their oviduct muscles to facilitate sperm transport, resulting in approximately 95 percent loss of the seminal fluid injected during mating (Baer, 2005). Sperm development occurs in the spermatheca, where the length of the sperm tail is critical for successful fertilization. Honey bees exhibit the longest sperm tails, which enhances fertilization efficiency (Slater et al., 2021). During their reproductive lifespan, queens store variable amounts of sperm and typically use two sperm cells per egg, resulting in strong sperm competition. Sperm received during mating can be retained for about 4 to 5 years, and queens can fertilize eggs using only about 1% of the stored sperm by the time of colony replacement (Baer et al., 2016). The quality of seminal fluid is innately linked to the developmental stage of the male drone (Rangel & Fisher, 2019). Drones raised in resource-limited colonies tend to produce less concentrated sperm, while those in well-resourced environments yield higher-quality sperm (Rousseau & Giovenazzo, 2016). The testes develop during embryogenesis before the initiation of spermatogenesis. The testis comprises approximately 150 testicular tubules, each containing undifferentiated germ cells, including primary spermatogonia (Lago et al., 2020).

3. Spermatogenesis in Honey Bees

During the final stage of larval development (5th instar), primary spermatogonia undergo four rounds of mitosis to produce sixteen secondary spermatogonia. Unique somatic cells encapsulate these secondary spermatogonia, ensuring the successful transport of semen throughout the maturation process (Lago et al., 2020). The secondary spermatogonia then proliferate several times within these cyst cells before transitioning into primary spermatocytes. This meiotic division, occurring during the late larval or prepupal stages, produces sperm. In the domesticated European honey bee (*A. mellifera*), spermatogenesis involves eight cycles, yielding approximately 202.8 spermatozoa per cyst cell (da Cruz-Landim C, 2001).

The seminal fluid consists of a range of components, including proteins, carbohydrates, metal ions, lipids, mucus gland secretions, and contributions from accessory glands (Colonello N.A. & Hartfelder K, 2003). The interaction between sperm and seminal fluid enhances sperm quality, prolongs storage, facilitates fertilization, and mitigates competition from other drones. Following emergence, there is a rapid increase in seminal fluid production, peaking at five to six days of age. This increase may depend on colony conditions, as worker bees provide care and nutrition to drones until they begin their mating flights (den Boer et al., 2010). During the Post-mating process, the queen receives both the ejaculate and its associated seminal fluid.

3.1. Morphological Variations in the Testicular Tubules during Spermatogenesis

Daily histological assessments conducted during spring and early summer enabled the study of testicular changes in drone bees from emergence to sexual maturity, revealing four distinct, overlapping spermatogenic stages. Stage I occurs between days 0 and 3, marking the conclusion of spermiogenesis. Stage II, spanning days 4 to 9, involves the expulsion of spermatoceles from the tubular lumens. Stage III spans days 7 to 13 and is characterized by progressive atrophy of tubular and follicular cells. Stage IV takes place between days 9 and 14, with seasonal variations noted that compress stages II and III; for example, July-raised drones reached maturity in early four days than those raised in June (Sawarkar & Tembhare, 2015). The testicular tubule serves as the locus of spermatogenesis, and variations in tubule morphology are expected to affect sperm production levels (Klein et al., 2021). Honey bee species such as *A. mellifera* have elongated testicular tubules and produce approximately 6-12 million spermatozoa per drone (Yániz et al., 2020). Testis size varies among drone populations and serves as a key differentiating feature of male genital organs. For example, different subspecies of *A. mellifera* exhibit size differences: *A. mellifera jemenetica* has a testicular volume of about 20.76 mm^3, while *A. mellifera carnica* measures at 30.43 mm^3, indicating a more robust testis (Schluns et al., 2003).

3.2. Regulatory Genetic Mechanisms in Spermatogenesis

Spermatogenesis occurs during the late larval and early pupal stages of development in drone bees (Tvedte et al., 2017). Critical meiotic genes in bees are essential for proper spermatogenesis, ensuring recombination and maintaining chromosomal integrity. Notably, the boule gene (bol) plays a central role in regulating spermatogenesis and facilitating the meiotic differentiation of sperm (Slater et al., 2021). Within the hymenopteran lineage, genes such as bol, bol1, bol2, and cdc25 are essential for optimal spermatogenesis. Disruption of these transcripts using RNA interference (RNAi) leads to the absence of mature sperm upon emergence (Sekine et al., 2015).

4. Variation in Sperm Quality in Drone Bee Population

The reproductive potential of drones is strongly dependent on sperm quality and quantity, as reductions in sperm viability can directly affect the queen fertility and colony success (Pettis et al., 2016). Research has identified two key factors affecting sperm morphology and fertility: sperm transfer efficiency and the sperm concentration required for oocyte fertilization. These factors are crucial for the effective production and preservation of seminal fluid (Yániz et al., 2020). The presence of sperm heads is a primary criterion for evaluating seminal fluid quality (Rhodes et al., 2011). Techniques such as ejaculation induction and seminal vesicle dissection have been employed to collect drone semen for analysis. In recent years, induced ejaculation has become the predominant method for obtaining seminal fluid samples (Cobey et al., 2013).

4.1 Semen Volume

Semen volume in honey bees is typically measured using either a syringe or a calibrated capillary tube. The yield from a single drone can vary significantly, ranging from 0.1 to 2.4 µL (Gencer & Kahya, 2011). Factors such as age, body weight, season, and genetic lineage can influence the volume of semen produced by individual drones (Bratu et al., 2022).

4.2 Quantitative Attributes of Sperm

Assessing quantitative attributes of drone sperm, particularly spermatozoa number and semen production, provides important insights into male reproductive potential in honey bees. Factors such as age, season, and genetic background significantly influence semen and sperm production, thereby affecting reproductive performance (Rhodes et al., 2011). Methods such as automated image analysis, flow cytometry, fluorescent plate readings, and standard cell counts are routinely employed to evaluate the qualitative and quantitative aspects of mammalian seminal fluid. However, their application in insect reproductive studies, including those involving bees, remains relatively limited (Anzar et al., 2009). The distinctive morphology of honey bee sperm, lacking the prominent head observed in mammalian sperm, complicates identification via image analysis. Integrating fluorescent microscopy with image analysis may allow for efficient and accurate identification of spermatozoa (Delaney et al., 2011). The maximum spermathecal capacity in the queen is approximately 9 million sperm heads, a critical factor in determining the total sperm quantity produced by individual drones and the sperm concentration in the whole seminal volume. Reports indicate significant variation in sperm production among different drone strains and species (Metz & Tarpy, 2019). Sperm yield varies among drones based on factors such as body weight, age, breeding season, genetic lineage, and health status. In particular, larger, more robust drones around 21 days old tend to produce the highest sperm counts (Rhodes et al., 2011). Discrepancies in spermatozoa counts reported by various studies may stem from methodological inconsistencies, inbreeding effects, and differences in drone rearing and maintenance environments (Kotze et al., 2024).

4.3 Quality factors of sperm contents

The assessment of potential and successful reproduction in drone bees can be inferred from the availability of seminal fluid upon the eversion of the endophallus. Drone bees have the advantage of direct transfer of seminal fluid to the spermatheca. While honey bees exhibit minimal to no sperm competition, drones that produce higher sperm counts are more likely to achieve fertilization (Page, 1986). Notably, drones inseminated with larger sperm quantities exhibit greater mating success than those with lower sperm quantities. Research indicates that smaller drones are associated with reduced reproductive success, mainly due to suboptimal sperm counts (Gençer & Kahya, 2019). Furthermore, the interaction of sperm, a factor which remains underexplored, plays a significant role in reproductive success. The seminal vesicles group sperm and associated particles, forming a thick seminal fluid. Variations in testicular tubule count, spermatogenesis quality, ejaculatory success, sperm migration dynamics from the testes to the oviduct and ultimately to the spermatheca, and sperm storage capacity contribute to the overall variability in sperm quality (Pizzari & Foster, 2008).

5. Impact of abiotic factors on the drone sperm viability

Viability of the drone sperm in both pre-mating and post-mating circumstances is influenced by various stresses in an ecosystem. The major abiotic factors that affect drone sperm viability are listed below.

5.1. Temperature

Temperature greatly affects the viability of stored seminal fluid to a great extent (McAfee et al., 2020). Incubating *A. mellifera* drones at low temperatures (32-35◦C) during the capped brood stage results in lower semen production but higher sperm viability. High temperatures during in vitro storage would considerably reduce drone sperm viability. The preserved drones would ejaculate around 40 per cent more dead sperm contents than freshly collected specimens (Czekońska et al., 2013). When *A. mellifera* young queens are transported, the temperature shifts occur at both high and low temperature prefendum, which tends to substantially reduce sperm viability in the spermatheca. High temperatures (45 ◦C) can kill almost 50 per cent of sperm in the genital chamber of the queen. The insemination process would be more efficient and effective at an average temperature of around (30- 35ºC). The temperature preference is crucial for maintaining the quality of drone sperm when storing it in vitro for specific periods (McAfee et al., 2019). The efficient transfer of seminal fluid usually takes place under low temperature conditions for all hymenopteran species (Liu et al., 2020).

5.2. Impact of Pesticides

Pesticides negatively affect drone fertility factors and the storage of inseminated seminal fluid in queens. Neonicotinoids were registered for more than 140 crops and used in more than 120 countries worldwide (Straub et al., 2016). Imidacloprid and thiamethoxam are the two commonly used neonicotinoids that have a severe negative impact on male bee sperm quality. The continuous exposure of thiamethoxam at 4.5 ppb reduced the life duration and sperm count of male bees by about 39 per cent. Drone sperm motility decreased after the high dose risk of imidacloprid (0.02 ppm), although there were differences in the per colony effect. Exposure of seminal fluid to imidacloprid (0.02 ppm) or coumaphos (100 ppm) significantly reduces the viability of seminal fluid by 33 – 50 per cent in the spermatheca of female bees (Inouri-Iskounen et al., 2020). Beekeepers commonly use pesticides such as Amitraz to control the honey bee varroa mite, *Varroa destructor* (Anderson & Trueman), the most significant pest of honeybees. The existence of sperm preserved in the laboratory settings in honey bee queens is unaffected by sublethal levels of Amitraz. However, it has affected development by downregulating genes associated with detoxification, cAMP-dependent protein kinase, immunity, and antioxidant capacity (Chaimanee et al., 2019). The honey bee drones showed decreased sperm concentration and motility, along with increased sperm metabolic rate, resulting in reduced fertility when exposed to Fipronil at 0.1 mg/L (Kairo et al., 2016; Belsky et al., 2020). Pesticides at low or nonlethal levels may not kill bees but may impair their reproductive ability (Straub et al., 2016; Gradish et al., 2019). To further understand the toxicity of neonicotinoids and their impact on male bee reproductive capabilities, several low or non-lethal amounts of pesticides of this class should be investigated for their effects on male bee sperm quality and foraging behaviour. Future research should evaluate the impact of several newer chemical substances commonly used by apiarists to prevent pests and diseases on colony maintenance on the reproductive capability of drone bees.

6. Impact of Biotic Factors on Sperm Viability

The production and quality of viable seminal fluid depend on several biotic factors such as age, stage, and physical and physiological conditions of drone bees.

6.1. Stage and Neonatal Weight

Research on the correlation between age-related factors and sperm production, maturity, and viability in honey bee drones has been conducted across various locations. Notably, male social insects cease sperm production upon eclosion (Rangel & Fisher, 2019). Age continues to influence sperm volume and concentration (Hayashi & Satoh, 2019). In honey bee drones, sperm becomes enveloped by nutritive cells as it transits from the testes to the seminal vesicles. Approximately 6–14 days post-mating, sperm tails elongate and reach maturity within the seminal fluid. This maturation process is accompanied by a decrease in seminal fluid volume, despite an increase in sperm density. Comparative analyses reveal that drones aged 14 to 21 days exhibit higher ejaculate volumes and sperm counts than their 35-day-old counterparts with similar sperm counts (Rhodes et al., 2011). Typically, sperm counts in the seminal vesicles can reach around 730,000 within three weeks post-emergence but decline sharply after 30 days. The viability of sperm and its concentration show a strong positive correlation with drone age (Metz & Tarpy, 2019). While sperm viability declines with age, motility patterns remain relatively stable (Locke & Peng, 1993). Variability within colonies may delay the onset of ejaculated sperm senescence, but factors influencing sperm viability tend to deteriorate with increasing drone age (Sturup et al., 2013). Sperm viability is not determined solely by drone age; age-associated alterations in chromosomal integrity, coagulation dynamics, and proteolytic activity also play important roles. In older drones, diminished protease activity compromises sperm motility and binding efficiency (Ben Abdelkader et al., 2014). Multiple studies indicate that 14-day-old drones exhibit markedly higher DNA fragmentation rates than younger cohorts, underscoring the need to evaluate sperm quality across male age classes. The age of both drones and queens is critical for successful mating and the establishment of progeny colonies across all bee species (Borsuk et al., 2018).

6.2. Body Size of the Drones

The lifespan and reproductive success of bees are intricately linked to their morphological and physiological attributes (Czekonska et al., 2018). Worker bees primarily produce drones from unfertilized eggs, resulting in a drone population of roughly 8% when a viable queen is present. In the absence of a queen, non-reproductive workers enhance vitellogenin expression. This mechanism is evident across various honey bee subfamilies that display differing oviposition success rates (Peso et al., 2015). Queen Right Colonies (QRC) typically produce drones that are physically and biologically superior compared to those generated by Laying Worker Colonies (LWCs). Drones from worker cells in LWCs tend to be smaller in size and lighter. Drone body size is measured by the length and width of their forewings, which correlate with sperm quantity. Larger drones (LDs) consistently exhibit significantly higher average sperm counts, larger accessory mucous glands, and more extensive seminal vesicle development compared to smaller drones (SDs) (Schluns et al., 2003). Smaller drones exhibit inferior physiological performance compared with standard-sized drones (Gencer & Kahya, 2011). Notably, the flight patterns of larger drones often align with those of the queen, facilitating mating opportunities (Couvillon et al., 2010). SDs exhibit reduced sperm competitive ability compared to LDs. Larger males tend to be overrepresented in progeny, while smaller drones are associated with lower paternal frequencies (Gençer & Kahya, 2019). Smaller drones exhibit lower activity levels, which further constrains their reproductive success, a trait closely linked to sperm concentration in the ejaculate. From an evolutionary perspective, drones have adapted to produce sperm in both large quantities and high concentrations.

6.3. Drone gland proteins

In honey bees, the activity level of ejaculated sperm surpasses that of sperm retained in the spermatheca, likely benefiting from seminal plasma proteins. These seminal components may enhance sperm viability, with *A. mellifera* semen capable of sustaining sperm life for up to 24 hours, thereby increasing its effectiveness (Poland et al., 2011). The structural integrity and functionality of seminal proteins are crucial for optimal insemination of queen bees (Baer et al., 2009). Seminal fluid proteins in honey bees have been identified using mass spectrometry, revealing differences in abundance among genetic lineages (Baer *et al*., 2012). Seminal fluid contains numerous proteins, including secretions from the accessory mucous and accessory glands, which enhance sperm motility and reproductive performance (Zhao et al., 2021). Several proteins present in the seminal vesicle overlapped with the proteins in the spermatheca. The seminal fluid proteins are responsible for maintaining sperm head integrity and facilitating efficient fertilization (Baer et al., 2009). While seminal fluid proteins are well characterized in many insects, their homologs are notably absent from the honey bee genome. This observation aligns with the unique mating system of honey bees and suggests that alternative molecular mechanisms mediate sperm storage and fertilization. Together with variations in sperm quality, viability, and protease activity among drones, these mechanisms play a crucial role in ensuring successful fertilization and colony reproductive success (Paynter et al., 2017; Bratu et al., 2022). Seminal fluids play significant roles in post-mating modifications of queen physiology and behaviour, influencing factors such as mating flights, ovary activation, ovulation processes, pheromone production, and transcriptional changes (Jasper et al., 2020).

6.4. Spermatheca proteins

Gland proteins in spermathecae have a wide variety of applications and are crucial to insect reproduction (King et al., 2011). The spermathecal secretory cells (SSCs) produce proteins and related substances needed for sperm preservation. Female reproductive fluid (FRF) can influence sperm characteristics, including motility, which may contribute to differences in sperm head performance during post-mating processes (Goenaga et al., 2021). Protective proteins found in the spermathecae of *A. mellifera* queens enhance the motility of the sperm and preserve the seminal fluid for long-term storage by the utilization of the spermathecal proteome derived from the vesicular glands (Poland et al., 2011). Spermathecal proteins also play a prominent role in immune responses and energy metabolism by synthesizing several enzymes (Baer et al., 2009). These antioxidant-encoding genes are upregulated in the spermathecae after mating to protect sperm from the oxidative damage caused by reactive oxygen species (ROS) (Gonzalez et al., 2018). Expression of the antioxidant genes TXN2 and TXNRD1 is higher in mated queens compared to non-mating queens (Santos et al., 2020).

6.5. The Role of Signalling Pathways

The MAPK/MAPKK pathway plays a pivotal role in regulating and activating kinase cascades, which are crucial for sensory mechanisms during drone mating flights (see Table 1). Research indicates that MAPK signalling is actively involved in reproductive and neural tissues, contributing to sensory integration in drones during mating (Ashby et al., 2016). Phosphoproteomic analyses reveal significant mTOR-related phosphorylation events during embryogenesis and testicular development, highlighting its critical involvement in spermatogenesis and protein synthesis as drones mature sexually. The Notch signaling pathway is instrumental in neuronal differentiation, particularly by shaping the antennal lobes, which are essential for pheromonal detection during mating flights within the DCA. Furthermore, it plays a crucial role in the behavioral maturation of drones during the eclosion process (Ma et al., 2022). Additionally, active Wnt pathway phosphoproteins and expression markers in drone embryogenesis and antenna gene sets illustrate the pathway influence on guiding axonal pathfinding and cytoskeletal dynamics mediated through β-catenin and Ca^{2+}-dependent mechanisms (Chen et al., 2017). The drone's seminal fluid impairs the queen's vision and reduces proximity during mating flights by altering gene expression in the brain and interfering with phototransduction (Liberti et al., 2019). Identical variations in gene expression are observed across honey bees, including post-mating queens, in several pathways such as phototransduction, neuroactive ligand-receptor interactions, Hippo signalling, and the phagosome pathway (Kocher et al., 2008).

7. Conclusion

This review delineates the biological and physiological characteristics of seminal fluid and its critical functions within various signalling pathways and glandular proteins that underlie efficient mating behavior and reproductive success in social insects, particularly honey bees. Despite the insights gained, the physiological roles of drones in maintaining genetic diversity require further exploration. Future research should delve into the intricate physiological processes that contribute to this phenomenon. The studies also elaborate on the numerous factors influencing sperm viability during mating and storage, ultimately facilitating efficient reproduction and colony maintenance. Moreover, the findings underscore the importance of seminal fluid in effective paternity care and in the production of viable queens, both of which are critical to the colony's longevity. This review aims to address existing knowledge gaps regarding the quality of honey bee seminal fluid, which has significant implications for various applications. It summarizes the glandular proteins and their candidate genes that are responsible for the signalling pathways necessary for optimal production and utilization of seminal fluid, ultimately supporting colony vigour in honey bee populations. Furthermore, this study addresses novel signalling pathways involved in effective sperm transfer and sperm motility, and the specific functional roles of proteins in the seminal fluid and spermathecal gland in efficient storage and use of drone sperm. It provides a comprehensive understanding of the molecular origins of variations within honey bee sperm components, facilitating appropriate emergence. Nonetheless, several unresolved questions remain regarding how drones generate and sustain their ejaculates throughout their lifespan, the impact of genetic variation on reproductive fitness, and methods to identify critical traits that evaluate drone fertility. Future investigations should focus on refining breeding programs and mitigating environmental stressors to enhance drone quality, thereby ensuring the stability and diversity of honey bee populations and bolstering the resilience of bee communities amid global environmental change.

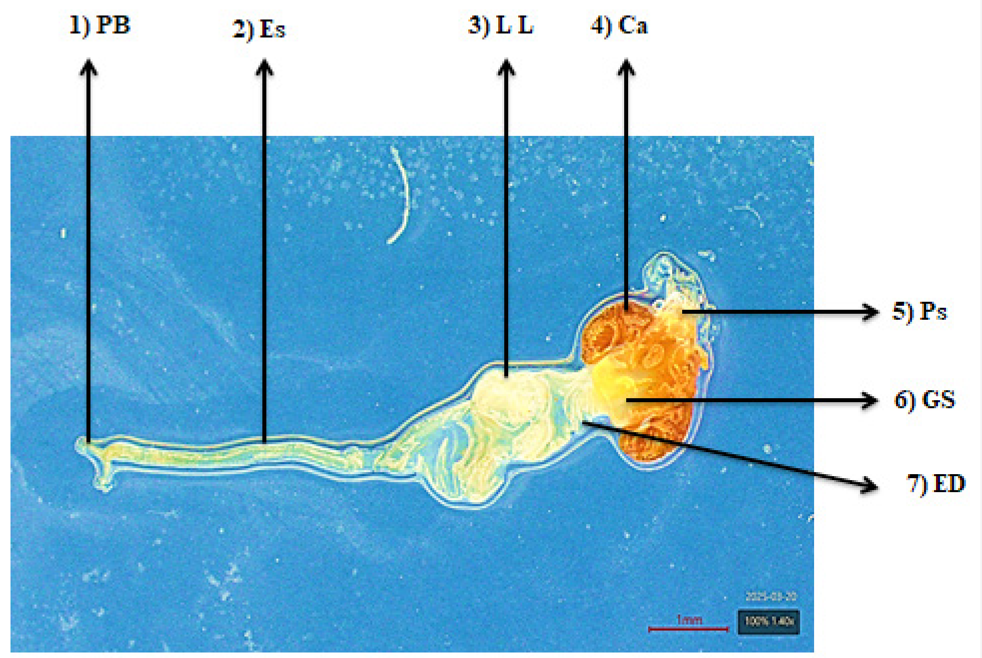

Fig 1. Male Genitalia of *Apis cerana indica* during the testis developmental stage. **1.** Penis Bulb (**PB**); **2.** Endophallus **(ES)** (Main Shaft); **3.** Lateral Lobes (**L L**); **4.**Cornua (**Ca**); **5.** Parameres (**Ps**); **6.** Claspers (or Gonostyli) (**GS**); **7.** Ejaculatory Duct (**ED**).

Fig 2. Endophallus eversion of *Apis cerana indica*. **1.** Cornua (**Ca**); **2.** Endophallus Shaft (**Es**); **3.** Seminal Vesicle **(Sv)**; **4.** Bulb or Basal Ring (**BB**).

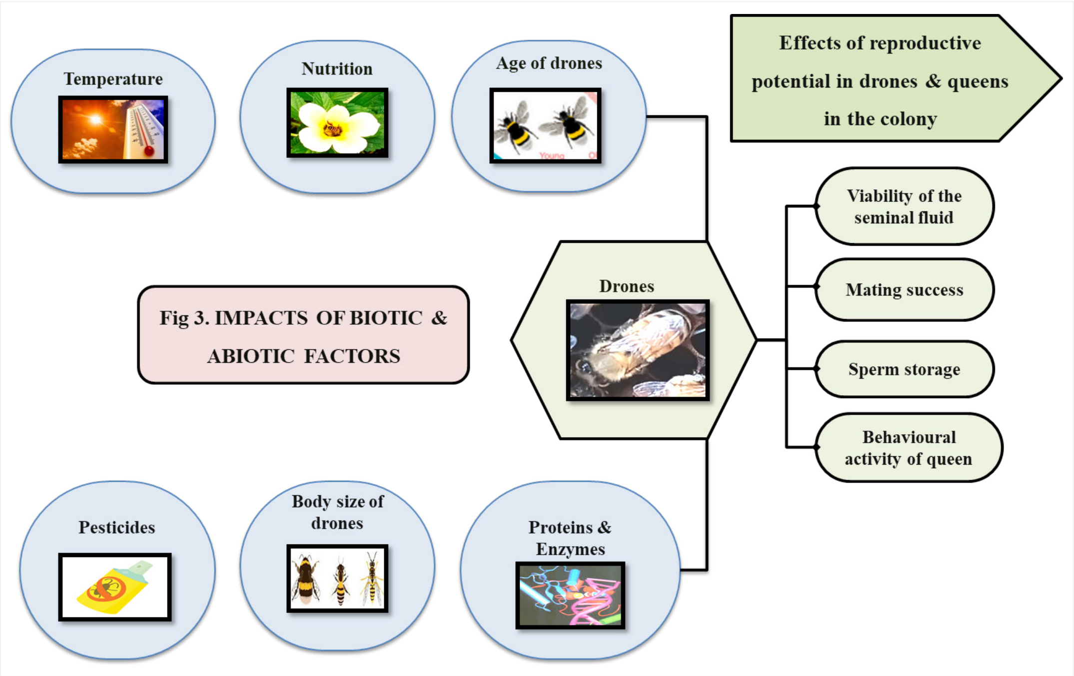

Fig 3. Impact of Abiotic and Biotic factors on Drone sperm viability.

Table 1. Major Pathways responsible for the critical regulatory functions in drone physiological system.

S. No	Regulatory Functions	Major Pathways involved	References
1.	Sensory processing, learning and ovipositional regulation	MAPK, Notch, Wnt signaling pathway	Chen et al., 2017
2.	Metabolic regulation, muscle development and behavioural gene signaling	MAPK, mTOR	Wang et al., 2009
3.	Reproductive tissue development and maturation	mTOR, Hippo, Wnt	Ma et al., 2022
4.	Aids in Reproductive physiology and Embryogenesis	Notch- Wnt- Hippo networks	Fang et al., 2014

Table 2. Major roles of male and female reproductive glands in social bees.

Reproductive part	Pivotal role	Illustration	Notable insect examples	References
Testicles of males	Retention and sperm transportation to viable females	In honey bee queens, gene expression within the ovarioles of the reproductive tract is modified that reflecting changes associated with reproductive activity. Semen has a distinctive capacity to keep sperm alive for up to several hours that cannot be duplicated by additional proteins.	*A. mellifera*	Baer et al., (2016) Lago et al., (2020) Colonello et al., (2003) McAfee et al., (2021)
Male reproductive Contributions	Promotes post-mating physiological and behavioral changes in queens	Reduce sexual receptivity of queens by altering the expression of vision-related genes in their brain.	*A. mellifera*	Delaney et al., (2011) Pizzari T. & Foster K.R, (2008)
	Regulates sperm competition and post-mating queen modifications	Directly benefit from previously preserved sperm in females.	*A. mellifera*	
Female Spermatheca	Long term storage and protection of viable sperm fluids	For antioxidant defence and energy metabolism of enzymes. Genes related to ion transport, signaling and localization are abundant in the seminal receptacle. The *mTOR* signalling pathway, amino acid production and *GPL* metabolism are enhanced.	*A. mellifera*	Klein et al., (2021) Pitnick et al., 2020
		Antioxidant enzymes including peroxidases, play a crucial role in protecting sperm from oxidative stress and supporting long-term fertility.	*A.cerana indica*	Kowalczyk A, 2022

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Authors' Contribution

K.R.N.: data curation, writing-original draft, writing-review & editing.
V.R.S.: conceptualization, writing-review & editing.
G.P.: conceptualization, writing-review & editing.
A.V.S.: conceptualization, writing-review & editing.
P.A.S.: conceptualization, writing-review & editing.
M.N.: writing - original draft, writing-review & editing.
P.C.P.: writing - original draft, writing-review & editing.

Conflict of Interest

The authors declare no conflict of interest during the study.

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