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Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review

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Abstract – In the honey bee, *Apis mellifera*, colonies are composed of one queen, thousands of female workers, and a few thousand seasonal males (drones) that are reared only during the reproductive season when colony resources are plentiful. Despite their transient presence in the hive, drones have the important function of mating with virgin queens, transferring their colony's genes to their mates for the production of fertilized, worker-destined eggs. Therefore, factors affecting drone health and reproductive competency may directly affect queen fitness and longevity, having great implications at the colony level. Several environmental and in-hive conditions can affect the quality and viability of drones in general and their sperm in particular. Here we review the extant studies that describe how environmental factors including nutrition, temperature, season, and age may influence drone reproductive health. We also review studies that describe other factors, such as pesticide exposure during and after development, that may also influence drone reproductive quality. Given that sperm development in drones is completed during pupation prior to adult emergence, particular attention needs to be paid to these factors during drone development, not just during adulthood. The present review showcases a growing body of evidence indicating that drones are very sensitive to environmental fluctuations and that these factors cause drones to underperform, potentially compromising the reproductive health of their queen mates, as well as the overall fitness of their colony.

Apis mellifera / drone / honey bee / miticides / pesticides / reproductive quality / queen

1. DRONE BIOLOGY

1.1. Development

Eusocial species in the order Hymenoptera are characterized by their haplo-diploid sex determination system, in which male and female development proceeds from unfertilized and fertilized eggs, respectively (Wilson 1971; Palmer and Oldroyd 2000; Collison 2004). Upon hatching, males of such species are typically nurtured in their colonies by sister workers until they reach sexual maturity (Stürup et al. 2013). Rearing males is costly, as they do not engage in any aspects of colony maintenance besides reproduc-

tion (Holldobler and Bartz 1985). Male rearing by workers is a common phenomenon among eusocial insects (Boomsma et al. 2005), particularly in swarm-founding species in the genus *Apis*, which are characterized by an extreme male-biased sex ratio among reproductives (Winston 1987; Baer 2005). In the honey bee *Apis mellifera*, a colony consists of a queen, several thousands of facultatively sterile female workers, and a few thousand seasonal males (drones). Drones are reared only during the reproductive season, which coincides with plentiful resources (Winston 1987; Rowland and McLellan 1987; Rangel et al. 2013) and a large worker population (Rangel et al. 2013; Smith et al. 2014). Production of drones is initiated by the construction of comb cells that are comparatively larger in size with respect to worker-destined cells (Seeley and Morse 1976;

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Boes 2010; Smith et al. 2014). There is, however, variation in the size of drone cells (Berg 1991; Berg et al. 1997; Schlüns et al. 2003), which results in differences in adult body size (Berg et al. 1997; Couvillon et al. 2010). In the annual cycle of a typical colony, drone production occurs 3 to 4 weeks before the production of new queens at the onset of the reproductive season, in a strategy presumed to maximize the access of sexually mature drones to virgin queens from nearby colonies during swarming (Page 1981). Unlike drones, which only mate once, honey bee queens exhibit extreme polyandry, mating with an average of 12 to 14 drones (Estoup 1995; Tapy and Page 2000; Rhodes 2002; Abdelkader et al. 2014), although extreme queen matings with 50 or more drones have been recorded (Palmer and Oldroyd 2000; Koeniger et al. 2005a, reviewed in Amiri et al. 2017; Brutscher et al. 2019).

Drone development from egg to adult emergence lasts approximately 24 days, exceeding the time of development for queens (16 days) and workers (21 days). This time frame can vary depending on factors such as haplotype (DeGrandi-Hoffman et al. 1998), temperature (DeGrandi-Hoffman 1993; Bieńkowska et al. 2011; Stürup et al. 2013) and overall colony condition (Winston 1987; DeGrandi-Hoffman 1993; Collison 2004). Like males in other Hymenoptera species (Holldobler and Bartz 1985), spermatogenesis in honey bee drones starts during the larval stage and concludes during the pupal stage (Bishop 1920; Hoage and Kessel 1968). Therefore, new adults emerge with all the sperm cells they will ever produce (Baer 2005). Estimates of the volume of semen ejaculates vary between 0.91 and 1.7 μL per drone (Woyke 1960; Nguyen 1995; Collins and Pettis 2001; Rhodes 2008; Rousseau et al. 2015), containing 3.6 to 12 million sperm cells (Mackensen 1955; Woyke 1962; Nguyen 1995; Collins and Pettis 2001; Duay et al. 2002; Schlüns et al. 2003; Rhodes 2011). Sperm counts appear to be strongly influenced by size, larval diet, and season (Nguyen 1995; Schlüns et al. 2003; Rhodes 2011). In the first week following emergence, sexual maturation in drones is completed by the migration of sperm to the seminal vesicles (Snodgrass 1956), along with the development of a pair of mucus glands that protects and provides

nourishment to the sperm (Woyke 1983; Rhodes 2008; Johnson et al. 2013; Rousseau et al. 2015). Sperm cells receive a measure of protection against pathogens from proteins contained in seminal fluid (Peng et al. 2016). The composition of such proteins is essential for sperm viability (Baer et al. 2009) and longevity (King et al. 2011). Interestingly, seminal fluid proteins implicated in immune responses are expressed at higher levels in drones infected with the microsporidian gut parasite *Nosema apis* (Grassl et al. 2017). Given the importance of the rearing environment during development on the reproductive quality of sexually mature drones, there is an urgent need to understand how environmental fluctuations during the rearing process may influence drone reproductive health.

1.2. Reproductive behavior

Climate, nutrition, and other environmental factors also affect the timing of drone sexual maturation (Rhodes 2008). Previous estimates of the age at which drones reach sexual maturity range from a low end of 6 to 8 days (Bishop 1920; Mackensen and Roberts 1948), to higher estimates of 10 to 12 days (Woyke and Ruttner 1958; Moritz 1989; Nguyen 1995), and even 16 days (Rhodes 2002) post emergence. For the first few days afterwards, young drones interact with workers near the brood area to be fed and groomed (Goins and Schneider 2013; Collison 2004). Orientation flights, which help drones learn the local landmarks and precise location of the nest, begin approximately 5 to 8 days following emergence (Tofilski and Kopel 1996; Collison 2004; Galindo-Cardona et al. 2015). Once a drone has learned the main landmarks and location of the hive, his life cycle culminates when he joins a drone congregation area (“DCA”) with a diameter of 30 to 200 m (Loper et al. 1987, 1992; Koeniger and Koeniger 2004), where as many as 11,000 drones gather midair at between 10 and 40 m above ground (Free 1987; Baudry et al. 1998; Koeniger et al. 2005a). Drones emit gland-produced odors that modulate social interactions among them (Villar et al. 2018) and likely aid in the formation of DCAs (Brandstaetter et al. 2014). When virgin queens enter a congregation area,

they attract drones with pheromones, in particular 9-oxo-2-decenoic acid (9-ODA; Brandstaetter et al. 2014), and by providing visual cues at short range (Gries and Koeniger 1996), which aid drones in finding and mating with queens (Baudry et al. 1998; Jaffé and Moritz 2010; Goins and Schneider 2013). Virgin queens typically visit DCAs on one or a few mating flights that can happen either in one or several days (Roberts 1944; Tarpy and Page 2000). DCAs are composed of drones from up to 240 colonies located up to 5 km away from each other (Free 1987; Baudry et al. 1998; Koeniger et al. 2005a). While this is the maximum flight distance recorded thus far, most drones tend to gather at DCAs located only a few hundred meters from their hive of origin, in a strategy presumed to maximize the amount of time they can spend at the DCA to increase their opportunity to mate (Koeniger et al. 2005b). Drones may also congregate near their own hives to avoid mating with related queens, given that most virgin queens fly several kilometers away from their colonies in search of mates, in a strategy presumed to help avoid genetic inbreeding (Winston 1987).

The criteria and environmental cues by which drones decide to gather at a given DCA are not well understood (Koeniger et al. 2005b). There is indication that factors such as vegetation structure, directionality, and density affect drone flight navigation (Galindo-Cardona et al. 2012, 2015). In one study, drones showed either enhanced or limited navigational abilities based on cardinal direction and distance, with some exhibiting higher rates of return to their colony from greater distances when returning from the north or south, but not having the ability to return from distances of 4 km or more when returning from an eastern direction (Galindo-Cardona et al. 2015).

Aggressive and territorial behavior appears to be absent among drones in DCAs (Koeniger et al. 2005a), although reproductive competitiveness does exist as a function of physical health attributes including symmetrical wing patterns (Jaffé and Moritz 2010; Metz and Tarpy 2019) and size (Berg et al. 1997; Schlüns et al. 2003; Hrassnigg and Crailsheim 2005). Shorter mating flights may also be indicative of underlying health issues such as heavy parasitism during pupation by the

ectoparasitic mite, *Varroa destructor* (Duay et al. 2002). Interestingly, Slone et al. (2012) showed that workers produce more trophallaxis-stimulating vibration signals toward drones that are perceived with poor flying capabilities, presumably to encourage the drones to be more competitive among each other.

Following copulation, the slender end of the endophallus (“mating sign”) breaks off and is pushed into the sting chamber in the queen’s reproductive tract (Koeniger 1990; Woyke 2008). Successful mating is fatal for drones, as they die soon after copulation due to dismemberment from ejaculating semen under great force by leaving the endophallus lodged in the genital tract of the queen (Page 1986; Woyke 2008; Goins and Schneider 2013). Queens bearing a mating sign elicit greater attraction from subsequent drone mates compared to queens lacking one. This indicates a method by which drones communicate the availability of a visiting queen that is still receptive to mating (Koeniger 1990), suggesting that cooperative behavior may occur among drones. Unmated drones typically live between 20 and 40 days post emergence (Page and Peng 2001; Stürup et al. 2013; Metz and Tarpy 2019) and are eventually evicted from the hive by the workers (Rhodes 2002). The determination to evict drones and halt their production is influenced by various environmental cues such as changes in temperature, time of the year, and reduction of food resources, particularly pollen (Rhodes 2002). Eviction occurs in the fall at the end of the reproductive season, when drones are no longer needed (Winston 1987).

Drones lack the structural modifications of workers (e.g., elongated proboscis and corbiculae) to engage in foraging behaviors (Hrassnigg and Crailsheim 2005). Even though drones may function in passive colony thermoregulation through their collective presence as part of a tight cluster (Fahrenholz et al. 1992), the exclusive function of drones is reproduction. As such, most studies examining drone biology and health have been done in the context of reproductive quality and ability. Below is an examination of the available studies that have explored various biotic and abiotic factors directly impacting honey bee drone reproductive quality.

2. FACTORS THAT AFFECT DRONE REPRODUCTIVE HEALTH

2.1. Effects of age, season and genetics

Environmental and biotic factors including age, season, and genetics can affect drone reproductive quality. Several studies in this area have found that drone senescence negatively affects sperm viscosity (Woyke and Jasiński 1978; Cobey 2007; Czekońska et al. 2013a), volume (Woyke and Jasiński 1978; Locke and Peng 1993; Rhodes et al. 2011; Czekońska et al. 2013a; Stürup et al. 2013), and viability (Locke and Peng 1993; Stürup et al. 2013). However, a few studies have not shown this to be the case, instead showing either constant (Metz and Tarpy 2019) or decreasing sperm viability over time (Czekońska et al. 2013a) in sexually mature drones. Interestingly, semen turns darker in color and becomes more viscous as drones age (Woyke and Jasiński 1978; Cobey 2007; Czekońska et al. 2013a). Semen from drones older than 21 days is too viscous, which makes it difficult for queens to expel excess semen from the oviducts, causing them to plug (Woyke and Jasiński 1978; Czekońska et al. 2013a). Furthermore, Locke and Peng (1993) found that aging affects drone sperm viability, which decreased to 86% in 14-day-old drones and 81% in 20-day-old drones. Likewise, Stürup et al. (2013) found that drones that survived more than 20 days post emergence exhibited as much as 50% lower sperm viability than those that survived less than 20 days. However, the impact of age on drone reproductive quality appears to be highly variable, and it is not always negative. For example, Metz and Tarpy (2019) recently sampled drones every day post emergence and found sperm viability to remain constant throughout life when correcting for sperm counts as drones aged. And, contradicting the previous studies, Czekońska et al. (2013a) found that, as drones aged from 15 to 30 days post emergence, semen volume decreased, while sperm viability increased (Table I).

Rhodes et al. (2011) not only explored the effects of age but also looked at the effects of season on drone semen volume and sperm counts. Drones examined at 14 and 21 days post

emergence yielded higher semen volumes than 35-day-old drones, while 21-day-old drones produced higher sperm counts than 14- and 35-day-old drones, which suggests that drone sperm counts peak at around 20 days post emergence, which was also what Metz and Tarpy (2019) found. In terms of season, Rhodes et al. (2011) observed a measurable seasonal increase in sperm counts, but a progressive decrease in semen volume, when transitioning from spring to autumn (Table I). In a similar study, Rousseau et al. 2015 observed an interaction between season and age on drone, given that semen volume was measurably different between 21-day-old drones and 14- or 35-day-old drones collected in spring and summer. Most importantly, they found that $87.8 \pm 6.2\%$ of the 35-day-old drones sampled released at least 0.2 μL of semen after manual eversion of the endophallus compared to the proportion of 14-day-old drones that released at least the same volume ($63.5 \pm 8.5\%$). Likewise, Zaitoun et al. (2009) found that drones of the Italian bee *Apis mellifera ligustica* produced more sperm (12.2×10^6 and 10.6×10^6 sperm cells, respectively) and were heavier in weight than those of the Syrian bee *Apis mellifera syriaca* (232 and 197 mg, respectively) in May compared to any other month (data not provided) between February and August for two consecutive years.

A colony's genetic structure also influences drone quality. For example, drones produced by laying workers in queenless colonies are typically much smaller and produce less sperm with more abnormalities than drones produced by the queen in queenright colonies (Gençer and Firatli 2005; Zaitoun et al. 2009). Different genetic lines also produce drones that differ in body weight, wing morphology, and sperm counts. For instance, Taha and Alqarni (2013) found that drones of the Carniolan bee, *Apis mellifera carnica*, were heavier and produced more sperm than drones of the Yemeni bee, *Apis mellifera jemenitica* (Table I). Yemeni drones also had testes, seminal vesicles, and mucus glands that were 47, 56, and 35% smaller, respectively, compared to Carniolan drones. Similarly, Rhodes et al. (2011) found that sperm numbers and ejaculation volume depend on a colony's genetic line. Of the four genetically distinct lines that the authors used (details not

Table 1. Summary of values obtained from studies that have explored the effects of age, season, and genetics on the reproductive health of honey bee drones

Study	Factor evaluated	Experimental treatments compared	Semen volume (μL)	Sperm counts	Sperm viability (%)	Percentage of drones that ejaculated semen (%)	Body weight or size (mg)
Czakońska et al. (2013a)	Age	15 days old	1.0 ± 0.2		87.8 ± 4.93		
		30 days old	0.9 ± 0.2		91.4 ± 3.12		
Rhodes et al. (2011)	Age	14 days old	1.0 ± 0.0	2.8 ± 0.1 × 10 ⁶ sperm cells			
		21 days old	0.9 ± 0.1	3.4 ± 0.2 × 10 ⁶ sperm cells			
		35 days old	0.8 ± 0.0	2.8 ± 0.2 × 10 ⁶ sperm cells			
Rousseau et al. (2015)	Age	14 days old				58.3	
		21 days old				65.9	
		35 days old				80.2	
Rhodes et al. (2011)	Season	Spring	1.0 ± 0.0	1.88 ± 0.14 × 10 ⁶ sperm cells			
		Summer	0.9 ± 0.0	3.12 ± 0.21 × 10 ⁶ sperm cells			
		Autumn	0.8 ± 0.0	4.24 ± 0.25 × 10 ⁶ sperm cells			
Rhodes et al. (2011)	Genetic line	1	0.7 ± 0.0	2.1 ± 0.2 × 10 ⁶ sperm cells			
		2	1.1 ± 0.0	4.1 ± 0.2 × 10 ⁶ sperm cells			
		3	0.8 ± 0.0	2.8 ± 0.2 × 10 ⁶ sperm cells			
		4	1.0 ± 0.0	3.1 ± 0.2 × 10 ⁶ sperm cells			
Taha and Alqarni (2013)	Haplotype	<i>A. m. carnica</i>		12.7 ± 0.0 × 10 ⁶ sperm cells			227.2 ± 0.6
		<i>A. m. jemenitica</i>		9.3 ± 0.0 × 10 ⁶ sperm cells			190.9 ± 0.33
Zaitoun et al. (2009)	Haplotype	<i>A. m. ligustica</i>		10.2 × 10 ⁶ sperm cells			203
		<i>A. m. syriaca</i>		8.8 × 10 ⁶ sperm cells			181

The values provided have been approximated to the nearest decimal place unless otherwise noted. See each citation for details on how the data were obtained

provided about the source from which the colonies came), drones from one line had higher semen volume and sperm counts than the other three lines, and drones in a separate line had significantly lower semen volume and sperm counts than the other three lines (Table I). Moreover, Fisher et al. (2018) recently examined drones from different apiaries during the summer in a limited geographic region around central Texas for two consecutive years. They found significant variation in sperm viability between sites, with some apiaries (and some of the colonies therein) showing significantly higher or lower sperm viability than others in the same area. However, these patterns were not consistent across years, suggesting that the genetic structure of each colony influenced drone quality only partially and that environmental factors such as forage availability and time of year (Kumar and Kaur 2003) likely influenced the variation in sperm viability between apiaries and across years more strongly than genetics. These studies show that the genetic composition of a colony influences many aspects of drone quality, including semen volume, sperm cell counts, and the proportion of drones that produce sufficient semen for proper insemination of queens.

In conclusion, there appears to be an overall negative effect of age on drone sperm viability (Woyke and Jasiński 1978; Locke and Peng 1993; Cobey 2007; Rhodes et al. 2011; Czekońska et al. 2013a) and seminal volume (Woyke and Jasiński 1978; Rhodes et al. 2011; Czekońska et al. 2013a; Stürup et al. 2013), but these patterns are not always consistent (Czekońska et al. 2013a; Metz and Tarpay 2019). Variable outcomes are also observed depending on the season (Rhodes et al. 2011; Rousseau et al. 2015) as well as the genetic lines of the colonies examined (Zaitoun et al. 2009; Rhodes et al. 2011; Taha and Alqarni 2013). Nonetheless, despite the influence of genetics, environmental factors may exert a greater influence on sperm viability, even among drones of similar genetic origins and geographical distribution (Kumar and Kaur 2003; Fisher et al. 2018). The inconsistencies in the results across studies point at the need for further research on the interaction effects between genetics and factors such as nutrition, location, age, and season, to more clearly determine the combinatorial influence of these variables on drone reproductive viability.

2.2. Effects of temperature and immunocompetence challenges

Under normal conditions, drones are reared in a brood nest area maintained at a constant temperature of 33 to 35 °C thanks to the thermoregulatory abilities of workers (Winston 1987). Not surprisingly, drone reproductive quality seems to be severely compromised when the temperature deviates from this tightly regulated range either during development, or after emergence. For example, Jaycox (1961) observed that sexual maturation in drones was hindered when they were reared at 31.1 °C instead of the optimal 33 to 35 °C range. Further, drones reared at 28.33 °C experienced a complete lack of sexual maturation (Jaycox 1961) and was entirely halted at 28.33 °C. Drones also lack a fully developed capacity to thermoregulate immediately upon emergence, probably because young drones exhibit a lower metabolic rate relative to mature drones (Abou-Shaara et al. 2017). Not surprisingly, sudden changes in temperature not only affect drone development but also reproductive quality. Bieńkowska et al. (2011) found that the number and viability of sperm cells transferred to a queen after mating were lower in drones kept at temperatures that were below or above an optimal temperature range. In particular, drones captured between 14 and 20 days post emergence were subjected to a suboptimal temperature of 9–10 °C, an optimal temperature range of 30–35 °C, or a high temperature of 40 °C for approximately 30 min before semen collection through manual eversion of the endophallus. Semen from all treatment groups was then used to artificially inseminate queens and to assess sperm number and viability between treatment groups. Even though no difference was observed in sperm number between drones subjected to the temperature treatments below and above the optimal range, drones subjected to 40 °C had significantly lower sperm viability, with sperm being dead in over 40% of the analyzed samples compared to 19 and 17% for the 9–10 and 30–35 °C treatments, respectively. Additionally, queens inseminated with semen from drones subjected to either 9–10 or 40 °C had lower viability and fewer sperm cells in the spermathecae compared to queens inseminated with semen from drones subjected to 30–

35 °C (Table II), indicating the importance of maintaining an optimal temperature range for increased sperm viability.

In another study, temperature stress (4 h at 39 °C) was followed by the drones being returned to their source hives for 24 h. Stürup et al. (2013) divided newly emerged drones into two treatment groups based on the temperature treatment they received. At 10 days of age, drones were collected every day for a week and were subjected to either a high temperature of 39 °C or an ambient temperature of 25 °C for 4 h. All drones were returned to their host colonies for approximately 24 h before being recaptured for semen collection. Semen collected from both groups was subsequently exposed to a high temperature (39 °C) or an ambient temperature (25 °C), upon which sperm viability was measured. Not surprisingly, exposure to the high temperature treatment significantly lowered sperm viability for both the heat-treated drones and the heat-treated semen. Furthermore, older drones ranging between 15 and 16 days of age exhibited greater resilience to heat treatments compared to 10-day-old drones. Similarly, Czekońska et al. (2013b) tested the effect of incubation temperature on drone quality by placing frames of pupating brood in incubators held at either 32 or 35 °C. The day before the expected emergence of drones, the frames were placed back into their respective colonies and assessed once the drones reached sexual maturity. The authors found that drones developing at 32 °C had larger testes, larger seminal vesicles, and larger mucous glands compared to drones that developed at 35 °C. Furthermore, drones that pupated in the incubator held at 32 °C exhibited higher sperm viability, but ejaculated significantly less semen with a lower sperm volume, compared to drones that developed at 35 °C (Table II). These results suggest that the sensitivity of drones to temperature fluctuations varies depending not only on the severity and duration of the temperature change but also on the developmental stage that the drones are in when subjected to the temperature treatment.

In conclusion, drones are highly sensitive to temperature changes, as sexual maturation may be entirely hindered by suboptimal rearing temperatures (Jaycox 1961). Being subjected to temperatures outside an optimal range even for short

periods significantly impacts the viability and abundance of sperm produced by drones (Stürup et al. 2013) and sperm stored in the spermathecae of queens (Bieńkowska et al. 2011). Being exposed to a higher-than-optimal temperature range also exerts a negative impact on the size of reproductive organs (Czekońska et al. 2013b).

Furthermore, while not much is known about the impact of injury on drone fitness, immune activation seems to incur a high cost for drones. To date, there has only been one study looking at the effects of injury and immunocompetence challenges on drone sperm viability. Stürup et al. (2013) collected sexually mature drones as they returned to the colony or attempted to take off on mating flights, and allocated them to one of two treatment groups. The first group was wounded using a hypodermic needle to puncture the inter-segmental membrane between the third and fourth abdominal tergites, while the second group was not wounded. Both groups were placed in an incubator at 33 °C before semen collection. Not surprisingly, wounded drones had significantly lower sperm viability than unwounded control drones, showing that the activation of the immune system to combat injury incurs a high reproductive cost to males. Even though proteins in the seminal fluid confer antifungal protection that promotes sperm viability and survival (Peng et al. 2016; Grassl et al. 2017), the immune responses that are triggered by external injury come at a high cost to the drones in terms of sperm quality, and potentially, longevity.

2.3. Effects of nutrition

Only a handful of studies have explored the effects of nutrition on drone reproductive quality, yielding mixed results. Czekońska et al. (2015) tested whether access to differing amounts of pollen during development affected drone reproductive quality. They used pollen traps to restrict colonies to only accessing an average of 932 g of pollen for the duration of the experiment, while control colonies had unlimited access to pollen over a 2-month period. Drones reared in colonies with limited pollen supplies had lower body weight, lower semen volume, and a lower probability of successful ejaculation when probed manually, compared to those with

Table II. Summary of values obtained from studies that have explored the effects of temperature on the reproductive health of drones

Study	Factor evaluated	Experimental treatments compared (°C)	Semen volume (μL)	Sperm counts	Sperm viability (%)	Percentage of drones that ejaculated semen (%)
Bieńkowska et al. (2011)	Temperature	10		$6.4 \pm 1.4 \times 10^6$ sperm cells	83	
		30–35		$7.1 \pm 1.2 \times 10^6$ sperm cells	88	
		> 40		$6.4 \pm 1.2 \times 10^6$ sperm cells	79	
Czekońska et al. (2013b)	Temperature	32	0.7 ± 0.3		85.9 ± 10.2	69.9
		35	0.8 ± 0.2		81.5 ± 10.7	88.2

The values have been approximated to one decimal place unless otherwise noted. See each citation for details on how the data were obtained

unrestricted access to pollen (Table I). However, no significant differences were observed in terms of sperm counts, viability, and concentration, regardless of whether the drones had limited or unlimited access to pollen.

In a similar study, Stürup et al. (2013) tested the effects of pollen deprivation on drone quality by collecting hundreds of emerging drones from three colonies and separating them into two treatment groups. Both groups of drones were maintained in small test colonies with unlimited access to 50:50 water:sugar solution. The colonies were then placed in flight cages that allowed workers to forage for nectar but prevented them from bringing pollen into the hive. One treatment group was only supplied with sugar water, while the other group was supplied with sugar water and an unlimited supply of pollen. Drone collection began when drones reached 12 days of age and continued over 5-day intervals until the drones were 22 days old, at which point the authors measured sperm viability. To do this, frames with drone pupae were placed in an incubator for 2 days before adult emergence, and emerged drones were caged and put back into their host colonies until they matured. No significant differences in sperm viability were found between drones reared with or without unlimited pollen supplies, similar to the results obtained by Czekońska et al. (2015). Similarly, Szentgyörgyi et al. (2017) deprived drone larvae from being fed by nurses for a period of 10 h during the second

instar or the fifth instar. They found that emerged adults were lighter in weight when the larvae were starved during either the second or fifth instar compared to larvae that were fed regularly during development (Table III). However, semen volume was not affected by pollen starvation.

Nguyen (1995) examined the effects of dietary supplements on drone health. The author found that pollen supplementation caused drones to become sexually mature 2 days earlier post emergence (at 10 days) compared to drones that were not fed supplementary pollen, which reached sexual maturity at 12 days post emergence, on average. However, pollen supplementation did not affect semen volume or sperm counts.

Rousseau and Giovenazzo (2015) found a positive effect of supplementing colonies with syrup and pollen in the springtime on drone quality. Drones reared on a diet supplemented with sugar syrup and protein patties were significantly larger upon emergence and had higher semen volume and higher sperm viability, than drones reared with no supplemental feeding (Table III), indicating that feeding protein and sugar supplements to drone-rearing colonies in the spring increases drone reproductive quality.

Reproductive quality may be influenced by access to sufficient pollen supplies, as drones reared in colonies with restricted pollen access experienced compromised reproductive potential through lower semen volume and reduce ejaculatory capabilities

Table III. Summary of studies that have explored the effects of nutrition on the reproductive health of honey bee drones

Study	Factor evaluated	Experimental treatments compared	Semen volume (μL)	Sperm viability (%)	Percentage of drones that ejaculated semen (%)	Body weight or size (mg)
Czekońska et al. (2015)	Nutrition	Unrestricted access to pollen	1.1 ± 0.8	80.0	80.0	262 ± 18.9
		Restricted access to pollen	0.9 ± 0.3	68.0	68.0	254 ± 20.3
Szentgyörgyi et al. (2017)	Nutrition	Larvae fed regularly by nurses				260.9 ± 2.01
		2nd instar larvae starved				254.1 ± 1.97
		5th instar larvae starved				239.4 ± 2.12
Rousseau and Giovenazzo (2015)	Nutrition	No supplemental feeding	1.1 ± 0.0	82.9 ± 0.4		240.5 ± 1.1
		Diet supplemented with sugar syrup and pollen patties	1.2 ± 0.0	79.7 ± 0.9		243.0 ± 1.4

The values provided have been approximated to the nearest decimal place unless otherwise noted. See each citation for details on how the data were obtained

(Czekońska et al. 2015). Other parameters of reproductive quality including sperm viability, count, and concentration, however, were apparently unaffected by pollen deprivation (Czekońska et al. 2015). Similarly, pollen deprivation did not negatively impact sperm viability when drones were maintained in semifield conditions (Stürup et al.). Semen volume, however, was similarly observed to be negatively impacted by protein deprivation (Szentgyörgyi et al. 2017). Nguyen (1995) found that pollen deprivation may contribute to delaying sexual maturation but did not find it to impact sperm viability or semen volume, though this latter measure is inconsistent with other studies. Colonies supplemented with pollen, specifically in spring, were observed to produce drones with higher sperm viability (Rousseau and Giovenazzo 2015). Other studies did not find an effect of pollen availability on sperm viability (Nguyen 1995; Stürup et al. 2013; Czekońska et al. 2015), indicating that there seems to be a significant interaction of season and protein availability on drone reproductive quality.

2.4. Effects of farmer-applied insecticides

The widespread use of pesticides in a variety of crop systems has contributed to an ongoing risk of

pesticide exposure for honey bees (Johnson et al. 2010; Ostiguy et al. 2019). Several studies have described adverse effects of worker exposure to various neonicotinoids including imidacloprid, clothianidin, and thiamethoxam (Aliouane et al. 2009; Schneider et al. 2012; Di Prisco et al. 2013). Exposure to thiamethoxam and clothianidin also showed negative effects on ovary size, lower sperm quantity, and sperm viability in the spermathecae of honey bee queens (Williams et al. 2015). Straub et al. (2016) recently examined the effects of exposure to thiamethoxam and clothianidin on the reproductive competency of drones. In their study, 20 colonies used for drone rearing were randomly assigned to either control or treated conditions. The queen in each colony was caged onto a drone frame first and then onto a worker frame 38 days after pollen paste feeding was initiated, so that drones and workers of approximately equal age were obtained at roughly the same time. The first 30 drones that emerged from each type of colony were checked to ensure that they were not parasitized with *Varroa* mites and were weighed, placed in hoarding cages with 20 workers, and fed pollen paste and 50% sucrose solution ad libitum. Colonies were fed either a combination of pollen, honey, and powdered

sugar that was free of neonicotinoids, or were fed the same pollen paste formulation with the addition of field-relevant doses of thiamethoxam (4.9 ppb) and clothianidin (2.1 ppb). Mortality was monitored daily for 14 days after caging the drones. Surviving drones in each cage were then removed and their seminal vesicles, testes, and mucus glands were dissected for sperm counts and viability analysis. Drones from colonies chronically exposed to thiamethoxam and clothianidin had significantly lower survival in the cages over the course of 14 days (16.8%) compared to drones in the control treatment (32.1%). Of the drones that survived after 14 days, no significant difference was observed in weight or sperm number between control and treatment groups (Table IV). However, drones in the treatment group lived longer on average (21 days post emergence) and had significantly lower sperm viability, than those in the control group, which lived only 15 days post emergence, on average (Table IV).

Another recent study looked at the effects of the common neonicotinoid insecticide imidacloprid on drone reproductive quality. Ciereszko et al. (2017) randomly fed 18 colonies either an insecticide-free pollen and sugar syrup paste or fed them a similar paste that contained 200 ppb imidacloprid. Workers were not prevented from foraging, but their colonies relied on the artificial food due to a lack of natural floral resources. Approximately 3 weeks after feeding on the supplemental diets, the queen in each colony was caged onto a drone frame for 24 h. Drones newly emerged from that frame were weighed, marked, and confined to the brood chamber using queen excluders. Marked drones were captured 15 days post emergence for semen collection through forced eversion of the endophallus. The authors found no significant differences in weight, sperm concentration, and mitochondrial membrane potential between drones in the control and the treatment groups. Sperm motility differed significantly between the control group and the 200-ppb treatment group, although, similar to Straub et al. (2016), significant colony effects were noted throughout the experiment, which could have resulted from the small drone sample size obtained per colony or from genetic differences between the colonies used in the studies.

Recent examinations of the systemic insecticide fipronil have also unveiled significant negative impacts on drone reproductive quality. Kairo et al. (2016) maintained drones in semifield conditions whereby they were fed sugar syrup containing fipronil. Drones continuously fed the contaminated syrup were captured 20 days post emergence to compare semen quality between them and drones fed an insecticide-free syrup. Fipronil exposure significantly reduced sperm viability and concentration, while it increased the metabolic rate of sperm, which is believed to contribute to drone infertility. Furthermore, queens inseminated with semen collected from fipronil-exposed drones contained sperm with significantly lower viability in their spermathecae compared to those inseminated with drones that were fed the insecticide-free syrup. Similar results were obtained for drones reared in laboratory conditions following exposure to fipronil in the sugar syrup, which experienced significantly lower sperm viability and concentration (Kairo et al. 2017a). Interestingly, the same research group found that fipronil interacts with the microsporidian parasite *Nosema ceranae* and their interaction causes lower drone sperm viability and antioxidant activity compared to untreated drones (Kairo et al. 2017b).

In conclusion, the few studies that have explored the effects of exposure to commonly used systemic insecticides in the food have consistently shown significant negative effects to drone reproductive quality by causing lower sperm viability in laboratory, semifield, and field conditions (Straub et al. 2016; Kairo et al. 2016, 2017a). Furthermore, the insecticide fipronil seems to synergize with, and exacerbate the severity of, infection with *N. ceranae*. It is important to note that, even though systemic insecticides are commonly used in many agricultural settings, neonicotinoids such as thiamethoxam, clothianidin, and imidacloprid, as well as the insecticide fipronil, are only occasionally found in concentrations above the level of concern (or even the level of detection) in hive products such as wax and pollen (Mullins et al. 2010; Traynor et al. 2016). Therefore, examinations of more pervasive in-hive pesticides present in wax and pollen may better expand our understanding of the pesticides that cause a more damaging effect to drone reproductive health.

Table IV. Summary of values obtained from studies that have explored the effects of farmer-applied insecticides on drone reproductive health

Study	Factor evaluated	Experimental treatments compared	Semen volume	Sperm counts	Sperm viability (%)	Body weight or size (mg)
Straub et al. (2016)	Food contaminated with agrochemicals	Colonies fed neonicotinoid-free pollen and sugar syrup paste		2.2×10^6 sperm cells	92.0	277.1 ± 17.1
		Colonies fed pollen and sugar syrup paste with 4.9 ppb thiamethoxam and 2.1 ppb clothianidin		1.5×10^6 sperm cells	83.5	278.3 ± 18.2
Ciereszko et al. (2017)	Food contaminated with agrochemicals	Colonies fed neonicotinoid-free pollen and sugar syrup paste				330.6 ± 21.5
		Colonies fed pollen and sugar paste with 5 ppb imidacloprid				322.9 ± 30.3
		Colonies fed pollen and sugar paste with 200 ppb imidacloprid				321.1 ± 30.7
Kairo et al. (2016)	Food contaminated with agrochemicals	Colonies fed fipronil-free sugar syrup	0.83 ± 0.11	10.5×10^6 sperm cells	69.7	
		Colonies fed sugar syrup with fipronil ($0.1 \mu\text{g/L}$)	0.85 ± 0.11	8.6×10^6 sperm cells	59.1	

The values provided have been approximated to the nearest decimal place unless otherwise noted. See each citation for details on data collection

2.5. Effects of beekeeper-applied miticides

Most managed honey bee colonies are exposed to multiple pesticides that are applied by beekeepers to control pests and pathogens that threaten honey bee health. The presence of some of these pesticides can be widespread, especially in colonies owned by commercial beekeepers, and can reach high concentrations in wax, honey, pollen, and bees (Mullins et al. 2010; Traynor et al. 2016). For over 25 years, *Varroa* mite populations have been controlled by beekeepers in the USA mainly with miticides, including the pyrethroid fluvalinate, which was introduced in the 1990s, and the organophosphate coumaphos, introduced in the 2000s (Rosenkranz et al. 2010). While they were efficacious initially, *Varroa* has developed resistance to both products nearly everywhere in the USA (Lodesani et al. 1995; Elzen et al. 2000; Elzen and Westervelt 2002). Since 2004, beekeepers began to use the formamidine product amitraz, and now most colonies have wax contaminated with high levels of amitraz and its derivatives (Corta et al. 2000; Mullins et al. 2010; Traynor et al. 2016). Due to the widespread use of these miticides and their persistence at high levels in wax, it is reasonable to expect that contamination of the wax used to rear brood in managed colonies may compromise bee health (Boncristiani et al. 2012). In fact, several studies have shown that the presence of miticides in brood-rearing comb negatively affects queen fertility by causing lower sperm viability in queen spermathecae, lower queen reproductive quality, and higher rates of supersedure (Pettis et al. 1991; Haarmann et al. 2002; Collins et al. 2004; Pettis et al. 2004; Rangel et al. 2013; Rangel and Tarpy 2015, 2016). Furthermore, our preliminary work has shown that the combined presence of fluvalinate, coumaphos, and amitraz in the queen-rearing wax significantly reduces a queen's egg-laying rate and her attractiveness to retinue workers (Walsh and Rangel, unpublished data). Miticide contamination of comb is also a serious problem for drone development, as it has been shown to reduce drone production (De Guzman et al. 1999), survival (Rinderer et al. 1999), sperm production (Fell and Tignor 2001), and sperm viability (Burley 2007; Burley et al. 2008; Fisher and Rangel 2018).

In the first study of its kind, Rinderer et al. (1999) evaluated the effects of *Varroa* mite infestation and the use of fluvalinate (active ingredient in the product Apistan®) on drone reproductive health. Fifteen colonies were created from packages of bees that had been treated with Apistan® and kept in a dark room for 5 days to remove any phoretic *Varroa* mites before installation. The packages were then placed into hives that had not come in contact with miticides. Five colonies were treated with strips of Apistan®, five colonies were left untreated, and five colonies received a frame of open drone brood from *Varroa*-infested colonies to increase mite numbers, but no Apistan® treatment. The number of *Varroa* mites was assessed from all drone frames as new adults emerged. The drones were subsequently marked and placed in an untreated colony until they reached 12 days of age, and were then recaptured and collected for dissection of the mucus glands and seminal vesicles. Drone mortality was recorded every day post emergence and flight times were recorded when drones were between 1 and 14 days old. One day after emerging, 97% of drones reared in colonies without *Varroa* mites or fluvalinate treatment were alive, while drone survival was 86.1% in *Varroa*-infested and 59.7% in fluvalinate-treated colonies. Mortality was higher in fluvalinate-treated colonies (66.9%) and *Varroa*-infested colonies (80%) when drones were between 12 and 18 days old, compared to drones in colonies that were neither treated with fluvalinate nor parasitized by *Varroa* (62.5%). Seminal vesicle weight, sperm counts, and flight times differed numerically but not significantly between both treatment groups and the control group. The pioneer assessment of drone sperm viability and flight duration by Rinderer et al. (1999) contributed greatly to our initial understanding of the implications of beekeeper-applied miticides on drone health and survival.

Years later, Burley (2007) explored the effects of miticides on drone reproductive physiology. Eight colonies were set up with naturally mated sister queens and supplied with frames of newly drawn drone comb to prevent pre-experimental pesticide contamination. Control colonies were left untreated, while experimental colonies were treated by hanging strips of either Apistan® (fluvalinate),

Checkmite+® (coumaphos), or Apilife Var® (containing thymol, eucalyptus oil and L-menthol) in between frames. Queens were confined to drone frames and these frames were transferred to a box above a queen excluder to trap emerging drones, which were marked and confined in the hive. Marked drones were captured and dissected for assessment of sperm counts and viability in the seminal vesicles when they were 14 to 20 days old. Drones in the coumaphos-treated colonies exhibited significantly lower sperm viability than control drones (Table V), but there were no differences in sperm viability between drones reared in control colonies and those treated with fluvalinate or thymol. Moreover, drone sperm counts were lower in colonies treated with fluvalinate, thymol, and coumaphos, compared to drones in untreated colonies. Not surprisingly, *Varroa* infestation levels were significantly higher in untreated versus treated colonies.

Burley (2008) further evaluated the effects of miticide exposure on drone sperm viability. Drones were reared in colonies that were randomly allocated to either an untreated group (control) or groups that were treated with fluvalinate, coumaphos, or thymol strips. Mature drones were captured from the hive entrance and their semen was collected in capillary tubes and stored for 6 weeks in a dark incubator kept at 25 °C to avoid sperm degradation. One pooled capillary tube from each experimental group was randomly selected and used for sperm viability analysis once a week for 6 weeks. Coumaphos-treated colonies produced drones with lower viability in the first week (86%) similar to drones in untreated colonies (90%), reaching the lowest viability by week 6, which was lower (49%) compared to untreated colonies (85%). Sperm viability during storage did not vary between drones in the control colonies and those treated with fluvalinate or thymol, however.

Johnson et al. (2013) explored the effects of in-hive miticides on drone survival and sperm viability. Drones were reared in plastic drone frames placed in recently re-queened colonies that had not been treated with either fluvalinate or coumaphos for at least 5 years. Frames containing drone brood were placed in the top box of a two-box colony above a queen excluder to prevent them from exiting the hive. Emerged drones were captured two to four days after emergence and were

topically treated with one miticide. Fluvalinate, coumaphos, amitraz, thymol, fenpyroximate, and oxalic acid were diluted individually in acetone at concentrations below the LD₁₀ rate for worker bees. Topical application consisted of dispensing approximately 1 µL of each miticide and acetone solution (or miticide-free acetone as a control) on the thorax of a young drone, which was then marked with a specific paint mark to indicate each treatment. All treated drones were returned to their host colonies and recaptured 2 to 3 weeks after treatment. Drone recapture was low across all colonies (14.8% of 6601 marked drones), including those only treated with acetone (14.7% of 1975 drones). There were no differences in sperm viability between drones in the control and any of the treated colonies (Table V). Interestingly, an important aspect of drone biology is the completion of spermatogenesis during development and prior to adult emergence (Bishop 1920; Hoage and Kessel 1968; Baer 2005). The exposure methodology used by Johnson et al. (2013) on adult drones was likely insufficient in assessing the effects of miticide exposure on drone sperm viability, given that the methods used did not accurately reflect the more field-realistic exposure to miticides by developing drones (instead of adults) being reared in contaminated beeswax.

Incidentally, Fisher and Rangel (2018) explored the effects of exposure of the drone-rearing beeswax to the five most ubiquitous miticides and agrochemicals found in hives, on the reproductive health of sexually mature drones. The authors hypothesized that, given that drones undergo spermatogenesis during development inside their cells and only undergo minor anatomical changes after emergence (Baer 2005), exposure to miticides of the brood-rearing wax would lower the quality of sperm once emerged drones reached sexual maturity. To test this, drones were reared in two consecutive years on plastic foundation frames coated with wax that was either pesticide-free (sprayed with acetone only) or sprayed with field-relevant concentrations of miticides including amitraz (4.3 mg/100 mL acetone) in the first year, or with a mix of coumaphos and fluvalinate (20.4 mg fluvalinate and 9.2 mg coumaphos/100 mL acetone), or the pesticides chlorpyrifos and chlorothalonil (5.4 mg chlorothalonil and 0.09 mg chlorpyrifos/100 mL

acetone) in the second year. Prior to emergence, the frames were placed in incubators with worker attendants, and once emerged, teneral drones were marked on the thorax and returned to their source colony. Twelve to 20 days after emergence, marked drones were captured and their sperm was collected for viability analysis. Average sperm viability was significantly lower in drones from all the treatment groups compared to those reared in the control, untreated wax (Table V). Interestingly, the authors also found that drones in the treatment groups reached sexual maturity when they were at least 16 to 18 days of age, similar to the findings of Metz and Tarry (2019), and a few days later than the 10 to 12 days post emergence that has been stated in conventional bee biology literature as the age at which drones reach sexual maturity (Winston 1987).

Shoukry et al. (2013) also assessed the effects of the miticides fluvalinate, amitraz, oxalic acid, formic acid, and thymol on drone health. Three colonies headed by naturally mated sister queens were randomly allocated to each treatment group, and miticide treatments were administered once the queens laid drone-destined eggs. Approximately 30 sexually mature drones were collected from each colony 14 and 20 days after emergence to assess parameters such as body weight, forewing length, and sperm counts. Drones from control colonies were heavier than those from colonies treated with all miticide treatment except for formic acid (Table V). Forewing length of drones from all miticide-treated colonies was shorter than those in control colonies (Table V). Finally, sperm counts were lower in drones from colonies exposed to fluvalinate and amitraz compared to any other treatment group or the control group (Table V), while a positive correlation was observed between sperm counts and wing length among all the miticide treatment groups.

In conclusion, even though insecticides widely used in the foraging environment can negatively impact drone reproductive quality (Straub et al. 2016; Kairo et al. 2016, 2017a, b), perhaps a more pressing threat is the presence of beekeeper-applied miticides used to control *Varroa* mites, which are still ubiquitous in a variety of hive products including wax, honey, and pollen (Mullins et al. 2010; Traynor et al. 2016). Miticides previously or currently used for *Varroa* control consistently show adverse effects on drone reproductive health

Table V. Summary of values obtained from studies that have explored the effects of miticides and/or agrochemicals on the reproductive health of honey bee drones

Study	Factor evaluated	Experimental treatments compared	Sperm counts	Sperm viability (%)	Body weight or size (mg)	Forewing length (mm)
Burley (2007)	Colonies treated with miticides	Untreated colonies	$6.0 \pm 0.3 \times 10^6$ sperm cells	98.5		
		Colonies treated with Apistan® (fluvalinate)	$5.3 \pm 0.2 \times 10^6$ sperm cells	Value not provided		
		Colonies treated with Checkmite+® (coumaphos)	$3.0 \pm 0.2 \times 10^6$ sperm cells	93.2		
		Colonies treated with Applife Var® (thymol, eucalyptus oil and L-menthol)	$4.8 \pm 0.4 \times 10^6$ sperm cells	Value not provided		
Shoukry et al. (2013)	Colonies treated with miticides	Untreated colonies	$5.4 \pm 1.4 \times 10^6$ sperm cells		211 ± 7	11.2 ± 0.3
		Colonies treated with Apistan® (fluvalinate)	$4.3 \pm 3.0 \times 10^6$ sperm cells		186 ± 10	10.6 ± 0.3
		Colonies treated with Mitac® (amitraz)	$3.5 \pm 1.1 \times 10^6$ sperm cells		186 ± 10	10.6 ± 0.3
		Colonies treated with oxalic acid	$4.7 \pm 1.8 \times 10^6$ sperm cells		188 ± 12	10.8 ± 0.2
Fisher and Rangel (2018)	Plastic drone frames sprayed with pesticides	Colonies treated with formic acid	$5.3 \pm 1.4 \times 10^6$ sperm cells		205 ± 16	10.9 ± 0.2
		Colonies treated with thymol crystals	$5.0 \pm 1.3 \times 10^6$ sperm cells		192 ± 23	10.8 ± 0.2
		Frames coated with pesticide-free wax sprayed with acetone only, year 1		99.2 ± 0.2		
		Frames coated with pesticide-free wax sprayed with 4.3 mg/100 mL acetone, year 1		80.1 ± 1.0		
		Frames coated with pesticide-free wax sprayed with acetone only, year 2		96.9 ± 0.6		
		Frames coated with pesticide-free wax sprayed with 20.4 mg fluvalinate and 9.2 mg coumaphos/100 mL acetone, year 2		80.0 ± 2.87		
		Frames coated with pesticide-free wax sprayed with 5.4 mg chlorothalonil and 0.09 mg chlorpyrifos/100 mL acetone, year 2		93.2 ± 1.16		

The values provided have been approximated to the nearest decimal place unless otherwise noted. See each citation for details on how the data were collected

(Rinderer et al. 1999; Burley 2008; Shoukry 2013; Fisher and Rangel 2018). This includes miticides that have persisted in wax and other hive components over a decade after their wide-scale abandonment by commercial operations due to Varroa's resistance to these products (Mullins et al. 2010). Interestingly, the timing of exposure is important, given that there is exposure to miticides during development, and therefore during spermatogenesis, is more damaging to drone reproductive health (Fisher and Rangel 2018) than exposure during adulthood (Johnson et al. 2013) potentially because a disruption of gametic production occurs during development (Bishop 1920; Hoage and Kessel 1968; Baer 2005).

3. CONCLUSIONS

Here we summarized the existing studies that have provided groundwork for our current understanding of how environmental and biotic factors affect honey bee drone reproductive health. Most of these studies show evidence that differences in age, temperature, season, haplotype, genetic line, and exposure to pesticides can negatively compromise drone sexual competitiveness. Interestingly, many of these studies report a delay in the age at which drones reach sexual maturation (Bishop 1920; Mackensen and Roberts 1948; Moritz 1989; Nguyen 1995; Rhodes 2002, 2008; Abdelkader et al. 2014; Fisher et al. 2018). Furthermore, while it was once believed that honey bee queens maintain only living sperm in their spermatheca (Woyke and Ruttner 1958; Ruttner and Koeniger 1971), more recent findings suggest dead spermatozoa are not entirely excluded from storage in the spermatheca (Collins 2000; Bieńkowska et al. 2011). Thus, drones experiencing a reduction in sperm viability or sperm count from a variety of factors (Rinderer et al. 1999; Rhodes et al. 2011; Bieńkowska et al. 2011; Stürup et al. 2013; Shoukry et al. 2013; Rousseau and Giovenazzo 2015; Straub et al. 2016; Kairo et al. 2016, 2017a, b; Fisher and Rangel 2018) may contribute a disproportionately high percentage of unviable sperm cells to a queen's spermathecal stores.

Queen replacement by workers (supersedure) often occurs when brood production falters (Hendriksma et al. 2004; Sandrock et al. 2014), which may happen if she is inseminated with an

insufficient amount of semen, or with poor quality sperm (Woyke and Ruttner 1976; Cobey 2007). Drones whose reproductive competitiveness is affected by extrinsic factors during development or adulthood may contribute dead or suboptimal sperm to a queen, which can have severe negative consequences not only for the queen herself, but for her colony's overall productivity and survival (Pettis et al. 2016; Kulhanek et al. 2017).

More attention needs to be paid not only to the factors that affect the reproductive quality of queens but also their mates, given that drones confer important contributions to the longevity of queens and the genetic diversity of the colony (Amiri et al. 2017). More research into the sublethal effects of the environmental and biotic factors faced by honey bee colonies need to continue to focus on how they affect drone reproductive health, which will help us develop better solutions to improve queen quality and overall colony health.

AUTHORS' CONTRIBUTIONS

JR and AF conceived this research, participated in the interpretation of data, performed analyses, wrote the paper, and participated in the revisions. All authors read and approved the final manuscript.

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Facteurs affectant la reproduction des abeilles mâles (*Apis mellifera*) - une synthèse

Apis mellifera / mâle / abeille domestique / acaricides / pesticides / qualité reproductive / reine

Faktoren, die die Reproduktionsfähigkeit von Drohnen der Honigbiene (*Apis mellifera*) beeinflussen - ein Übersichtsartikel

Apis mellifera / Drohne / Honigbiene / Milbenpestizide / Pestizide / Reproduktionsqualität / Königin

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