

# Breeding Focus 2021 - Improving Reproduction

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# Preface

“Breeding Focus 2021 – Improving reproduction” is the fourth workshop in the series. The Breeding Focus series was developed to provide an opportunity for exchange between industry and research across a number of agricultural industry sectors. With this goal in mind, workshops have included presentations across multiple agriculturally relevant animal species to take participants outside their area of expertise and encourage them to think outside the box. Reproduction is a main driver for profitability and genetic gain. We will discuss existing knowledge, identify gaps and explore genetic and management strategies to improve reproduction further in multiple species.

Successful reproduction is a complex characteristic comprising the formation of reproductive cells, successful mating and fertilisation, embryonic and fetal growth and eventually a successful birthing event. In livestock species, reproduction traits have mostly low heritabilities, which makes it challenging to improve reproduction as part of a multiple trait breeding objective. The complexity arises not just from the cascade of processes required to result in successful reproduction, but the relevant traits are different in males and females and they are influenced through health and fitness, nutrition, climate and other environmental and management factors.

Challenges to the improvement of reproduction can vary widely for different species. For less domesticated species such as abalone, the ability to produce and reproduce the animals in captivity presents a major challenge. In bees, reproduction has not been given great attention and little research has been undertaken to understand the underlying genetics of drone and queen reproduction. However, in all industries reproduction is recognised as the basis for genetic and economic gain. It directly influences the selection intensity that can be applied. It also determines how many animals are not required for replacement and can be sold. In all industries, irrespective of the challenge, cost-effective and easy to measure phenotypes of reasonable heritability are central. New technologies and approaches enable the development of novel phenotypes for genetic improvement which will be combined with a growing amount of genomic data in livestock species and together these developments provide new and exciting opportunities to improve reproduction further.

We would like to thank everyone who has contributed to this event for their time and effort: the authors for their contributions to the book and presentations, the reviewers who all readily agreed to critique the manuscripts. We would like to express a special thanks to Kathy Dobos for her contributions into the organisation of this workshop and the publication. Thank you!

Susanne Hermesch and Sonja Dominik

Armidale, May 2021

# Breeding for improved fertility of honey bees

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## Abstract

Honey bee (*Apis mellifera*) colony productivity and fitness is dependent on queen and drone quality, a culmination of the larval rearing environment, sexual selection and beekeeper-driven trait selection and management. Selection for both production and fertility traits of honey bees is not widely practised across commercially managed populations as it is in other livestock species. Scant research has been undertaken on drone and queen phenotypes, reproductive productivity and performance as it relates to selection for fertility traits. The opportunity for increased hive productivity through maximising fertility traits, in tandem with established commercially important colony level traits in honey bees exists globally. In this review, research on the characterisation, heritability, and breeding of known fertility traits of honey bees is discussed and recommendations are given on the most practical candidate traits for selection.

## Introduction

Honey bees are vital for global food security and contribute \$14 billion AUD per annum to Australian agriculture and horticulture industries through pollination services (Karasiński, 2018). The Australian beekeeping industry, however, is maintained primarily by honey production as its main source of income, not pollination. In order to sustain a productive industry, selection for economically valuable traits must be prioritised. Valuable traits such as pest and disease resistance, seasonal population growth and honey production are characterised as being moderately to highly heritable (Oxley and Oldroyd, 2010). Few of these traits have been continuously and simultaneously selected for in Australia and subsequent improved stock disseminated widely. Internationally, systematic selection has primarily been undertaken in populations managed for research, for traits such as hygienic behaviour (Spivak and Reuter, 1998; Spivak and Reuter, 2001), *Varroa* sensitive hygiene (Büchler et al., 2010; Danka et al., 2011) and pollen hoarding (Hellmich et al., 1985; Page and Fondrk, 1995). Selection for production traits, such as honey production and population growth, which underpin industry viability has been neglected. Productivity trait characterisation and heritabilities in honey bees have been

described, with established selection methods making meaningful genetic improvement possible for these traits within commercially managed populations (Brascamp, 2016). The opportunity also exists, given the variability in honey bee fertility traits, to include these heritable traits in breeding programs (Büchler et al., 2013; Rhodes, 2011).

While fertility trait selection is not systematically practised in large-scale honey bee breeding programs, brood viability is an exception. This trait indicates the overall fitness of a queen, how well-mated she is to an average of 12 or more drone sires with diverse sex alleles (complementary sex determiner) to herself, overall colony fitness, pest and disease resistance, and capacity for honey production (Oxley and Oldroyd, 2010). Brood viability is the top fertility trait candidate for inclusion in breeding programs. The weak history of record keeping across the industry and the unique method of reproduction of the species complicating the generation of estimated breeding values (Brascamp, 2014) are no longer the top barriers to increased selection, considering ongoing advancements in data collection and analysis. Prioritisation of time and cost of staffing for systematic record keeping as well as the will to initiate and commitment to maintain record keeping are the major barriers.

Recording of many queen and drone fertility traits is time consuming, expensive, impractical and is destructive if recording requires the death of the queen and drones. Fertility traits such as queen weight, overall size and brood viability, which are non-destructive and may have the greatest return on investment, should be prioritised for inclusion in bee breeding programs. In this review the additional fertility traits described, which are currently expensive and impractical to measure at scale, provide further support for a focus on weight, size and brood viability given their strong association with internal fertility traits.

## **Queen bee (dam) fertility traits**

The queen is functionally the sole reproductive female in the colony. She influences commercially important colony level production traits such as pollen hoarding and production of honey, propolis, royal jelly, and wax. This influence is primarily a result of her genetic contribution and fertility traits such as mating quality, brood viability, body and reproductive organ size, fecundity (i.e. egg production capacity and daily laying rate), and pheromone production. The queen's genetic contribution is via eggs and stored sperm from the multiple, haploid drone sires she mated with early in her adult life. She releases sperm when an egg is to be fertilised, yielding the diploid worker population. Through complex interactions between worker subfamilies (the offspring of different sires), the queen and the environment, measurable colony-level phenotypes arise (Brascamp et al., 2016).

Critical to queen quality is the age at which a female larva is transferred (grafted) into a queen rearing colony and initiation of royal jelly feeding triggers physiological changes from the worker to queen bee development pathway. Investigations on the variation of queen grafting age show queens raised from 1<sup>st</sup> instar fertilised larvae are higher quality adults, with greater body size, longevity and egg-laying capacity, than queens raised from fertilised larvae over

24 hours old (Büchler et al., 2013; Tarpy et al., 2011; Rangel et al., 2016). The larval rearing environment and queen rearing methods must be managed to the highest recognised standard methods as a prerequisite to fertility trait selection (Büchler et al., 2013). Any alterations to this standard, such as larval age at grafting, queen rearing method and environmental variants should be recorded so this information is available for modelling. Measurement of reproductive organs such as the ovaries and spermatheca are fatal, so evaluation through associated traits such as external queen morphological characteristics including adult weight and overall body size and brood viability are practical inclusions for commercial breeding programs.

Queen fertility traits are strongly linked to reproductive physiology which, while greatly affected by the larval rearing environment, may also be influenced by genetic effects (Rangel et al., 2016). Selectable fertility traits in queen bees include adult body weight, which is associated with ovary and spermatheca (sperm storage organ) size, and overall body size, which is associated with queen attractiveness to drones at mating flights, mating success, fecundity, and brood viability.

### ***Queen weight and overall size***

Queen weight and size is in part controlled by the age at grafting of the female larva, the larval environment, nutrition and genetics. Queens which are highly fecund and long-lived are generally larger in overall body size, mate with more drones and have greater sperm storage capacity (Tarpy, 2011). The larval diet fed to females can cause distinctly different phenotype development (polyphenisms) based on differential gene expression (Evans and Wheeler, 1999; Evans and Wheeler, 2000), influencing whether the larva will develop into a sterile female worker or a reproductive female queen. The beekeeper controls this outcome through environmental manipulations including colony management, supplemental feeding and grafting age.

Queen weight at adult emergence is a non-destructive, measurable fertility trait which shows promise as an individual level trait which influences colony level honey production (Costa Maia et al., 2018, Nelson and Gary, 1983). Queens which are larger in size at emergence also store larger numbers of spermatozoa in their spermathecae, the organ that stores semen from multiple males after mating, have greater submissive response from workers (retinue response) or attractiveness, and mate with significantly more males (Tarpy, 2011; Rangel, 2016); all individual level queen quality measures which contribute to colony fitness. Significant correlations between queen weight at emergence and colony quality, defined by honey production and sealed brood area, have been shown (De Souza et al., 2013; Nelson and Gary, 1983).

However, uncertainty remains as to the most appropriate age at which queen weight should be measured. Nelson and Gary (1983) weighed mated queens at 18 hours after removal from their mating nucleus colonies, 8 days after storage and transit, and at the peak of a honey flow, however, nominating 12 days post-emergence as a “practical method of eliminating some of the less productive queens.” At 12 days post-emergence, queens are still in their small mating colonies and not yet transferred into a production colony, making this weight point a potential labour saving and production increasing measure in the long term. Kahya et al. (2008) importantly



showed weighing at emergence had no negative effect on the timeline to mating and onset of egg-laying. A significant correlation ( $P < 0.01$ ) with queen weights at emergence to three days after emergence ( $r = 0.572$ ), at onset of egg-laying ( $r = 0.440$ ), three days after egg-laying ( $r = 0.562$ ) and one month after the onset of egg-laying ( $r = 0.808$ ) was shown (Kahya et al., 2008).

In the context of an artificial insemination program, queen weight at emergence or 7 days post-emergence, are the most practical assessment points given queen husbandry or insemination tasks occur at the same time respectively. Additionally, significant correlations between weight at emergence and spermatheca diameter ( $r = 0.610$ ) and volume ( $r = 0.607$ ) support weight at queen emergence (Kayha et al. 2008). While the highest correlation was weight at emergence with weight one month after egg laying ( $r = 0.808$ ), this time point is impractical given the recommended removal of a mated queen from her colony for sale starts at 21 days post emergence (Rhodes, 2011) and any further delay impacts on a commercial queen producers' income generation. Additional targeted research into correlations between age at post-emergence weight and queen and colony phenotypes is needed.

### ***Ovariole quantity***

Larval age at grafting and the rearing environment influence the development of hypertrophied reproductive organs in queen bees (Büchler et al., 2013). The development of ovaries, made up of ovarioles, has had particular focus by Hepperle and Hartfelder (2001) and Kahya et al. (2008). Ovariole quantity reaches its highest potential when female larval grafting age is between 0 to 24 hours post-emergence as a larva. After this it decreases with time. High ovariole quantities provide a greater capacity for egg production with the flow on possibilities of maximising daily egg-laying rate, colony population growth rate, subsequent production and overall colony fitness.

Although ovariole quantity is the preferred measure of ovary quality over ovary weight, which varies according to both the quantity of ovarioles and the seasonally variable, resource-dependent quantity of eggs and their stages of development within each ovariole (Kahya et al., 2008), this measure requires fatal dissection. Additional research into ovariole quantity and its relationship to queen weight at the timeframes already mentioned, would be useful. If they are correlated, queen weight as a proxy for destructive ovary dissection would be the preferred and more practical measurement.

### ***Spermatheca size and storage capacity***

Spermathecal measurements require unnecessary fatal dissection of the queen, given the significant correlation between the weight at queen emergence and the diameter of the spermatheca ( $r = 0.619$ ) (Kahya et al., 2008) and the correlation of queen weight and spermatheca volume  $r = 0.548$  ( $n = 38$ ,  $P = 0.0004$ ), is also highly significant (Collins and Pettis, 2013). While spermathecal size impacts spermatozoa storage capacity, the queen's larval diet or environment (De Souza 2018), availability of ample sires maintained in optimal temperature ranges, and con-

ditions within which the newly mated queen is cared for all impact on spermatozoa migration into the spermatheca (Ruttner and Koeniger, 1971; Woyke and Jasinski, 1973; Bieńkowska et al., 2011). Spermathecal size alone does not guarantee the quality of the mating or subsequent quantity of sperm given these effects. Additionally, standard methods for quantifying spermatozoa within the spermatheca are all destructive and under debate as to which is the most accurate (Koeniger, 2005), further supporting queen weight as a proxy for dissection given the significant correlations between queen weight and spermatheca size and volume.

### ***Queen pheromone production***

The queen's production of pheromones affects the behaviour of her worker progeny and subsequent colony-level traits. Queen pheromones influence her attractiveness to drones during mating, the colony's propensity to swarm, rearing of replacement queens, the age at which workers begin foraging and foraging activity (Oxley and Oldroyd, 2010; Rangel et al., 2016) and reproductive potential and suppression of the development of worker ovaries (Hoover et al. 2003). Research into queen mandibular pheromone requires destructive sampling and primarily confirms that controlling the larval age at grafting and rearing environment has substantial impact on queen pheromone production (Rangel et al. 2016). Queen mandibular pheromone production affects colony productivity and therefore profitability, however, testing methods are expensive and workers within a hive naturally replace queens with poor pheromone production rendering testing for pheromone production impractical for industry adoption.

### ***Fecundity - brood viability***

Brood viability is measured by visual assessment of the percentage of viable brood within the colony, generally scored on a scale or by measuring the area of viable brood. A measure of queen fecundity, high brood viability is the culmination of extreme polyandry, virgin queen fitness and complementary sex determiner allele diversity represented in available sires within the drone congregation environment and subsequently in the mated queen's spermatheca. De Souza et al. (2013) found a significant positive correlation between queen body weight at emergence, fecundity and colony quality. Soller and Bar-Cohen (1967) found a genetic correlation of 1.12 between total brood area and total honey production, citing an estimate greater than 1.0 due to sampling variation. They suggest brood area measurements prior to a major nectar production could serve as a proxy measurement for honey production. This hypothesis should be tested in diverse biogeographic regions within Australia where variable nectar production events from endemic species occur. Soller and Bar-Cohen (1967) estimated the heritabilities, calculated from variance components, of total brood area for winter and spring and brood area before the start of a major nectar production event of 0.76 and 0.33 respectively, however, more current estimations with a larger sample size are recommended.

Soller and Bar-Cohen (1967) indicate that the queen's daily egg-laying rate during the period before a nectar production event has the greatest influence on brood area and colony level honey production. The study did not include estimation of heritabilities of the queen's rate of

egg-laying but supports optimal rearing practices and selection for queens with higher overall body weight and likely greater quantities of ovarioles for increased daily laying rate and brood production. An increase in brood viability record inclusion in breeding programs is highly recommended as it minimises inbreeding and increases brood viability leading to greater profitability.

### ***Predisposition for drone production***

It is a function of queen bee biology that later in life she has a propensity to lay greater quantities of unfertilised eggs which develop into drones. In the author's experience and anecdotally, there is also variation between older queens' propensity to lay unfertilised eggs yielding drones (Joe Horner, personal communication, 1 February 2018), potentially having substantial impact on bee breeding program work plans. Further research is needed into the queen's propensity to lay unfertilised drone eggs, the colony's propensity to support greater numbers of drones, the heritability of these behaviours and seasonal effects on egg-laying and care propensities in queens and workers respectively, in order to maximise the bee breeders' ability to have ample sires available for uncontrolled mating or for semen collection in controlled mating.

### **Drone (sire) fertility traits**

Drones influence a colony's reproductive fitness by successfully mating with a queen and contributing genes to the next generation (Oxley and Oldroyd, 2010). As the neglected caste within research and breeding, drone fertility traits are generally not actively selected for, despite their reproductive success being a potential major proponent of natural selection in honey bees (Kraus et al. 2003).

Taha and Alqarni (2013), Rhodes et al. (2011), Fisher et al. (2018), Kumar and Kaur (2003), Rousseau et al. (2015) and Zaitoun et al. (2013) show that drone fertility traits, specifically semen volume and sperm viability, are affected by both genotype and environment. Rhodes (2011), Rangel and Fisher (2019), and Yáñez et al. (2020) reviewed the available literature on drone reproductive health measures and factors affecting them, including genetics. Known fertility traits in drones include body weight, age to sexual maturity, senescence onset, genital morphology, and sperm competition due to phenotype, motility, sperm production and semen volume. Described management factors affecting drone quality for fertility traits include the larval environment, age at mating, pesticides in the external or internal hive environment, and parasite loads (Rangel and Fisher, 2019). Ideally these management factors are known and controlled to ensure fertility traits can be measured accurately. Abiotic factors affecting drone quality during semen collection for artificial insemination include sperm processing and storage which, when undertaken with good hygiene and using standard protocols (Cobey et al., 2013) will have negligible influence on sperm viability or motility. Selection for fertility traits, particularly semen volume, sperm viability, drone longevity and any nonfatal proxy measurements for these traits, could be advantageous for the majority of queen breeders who do not control queen mating, where mating occurs in areas of unknown unmanaged colony population density

and phenotypes. Selection for drone fertility traits could both increase their reproductive competitiveness and decrease the generation time needed to make both fertility and production trait increases through the commonplace “open-mating” system of queen production in which mating is not fully controlled. Metz and Tarpy (2019) showed that drone reproductive physiology is more highly variable than previously thought, thus the need for further research into related traits. Heritability of currently described drone fertility traits are not known.

## ***Weight***

Different genetic lines, parasite pressures and rearing practices produce drones differing in adult body weight at emergence and sexual maturity, which is a contributing factor to reproductive competitiveness (Berg et al. 1997; Jarolimek and Otis, 2001; Schlüns et al. 2003; Hrassnigg and Crailsheim, 2005). There is a correlation between body weight and spermatozoa quantity, with larger drones producing more spermatozoa than smaller drones (Rinderer et al. 1985; Schlüns et al. 2003). Rinderer et al. (1985) compared weights of “young, apparently freshly emerged drones” collected within Africanized and European field colonies with spermatozoa counts from drones collected on their return flight from unsuccessful mating to their field colony. Schlüns et al. (2003) manipulated rearing practices to produce both normal and small size drones which were sexually mature when analysed. In addition to producing more spermatozoa larger drones also have greater reproductive success than smaller drones (Berg et al. 1997). Management factors and selection can be employed to maintain large drone body size and associated reproductive competitiveness (Büchler et al., 2013).

## ***Sperm production***

Koeniger (2005) previously cautioned against general conclusions about differences in spermatozoa present in the semen load, citing the possibility of errors in counting between multiple studies. Rhodes (2011) echoes Koeniger’s concerns, arguing that large differences in sperm counts may be due to the semen source, either ejaculate or seminal vesicle loads (Woyke, 1960; Schlüns, 2003; Anderson, 2004). Additional caution toward spermatozoa estimation is provided by Mazeed and Mohanny (2010) given their findings, albeit in a small sample size, that older drones had fewer total spermatozoa in all reproductive organs than younger drones.

Nonetheless, selection for greater sperm production is possible. Rhodes’ thesis (2011) notes season and age effects must be accounted for, finding drones across four distinct genetic lines produced significantly more sperm when reared in autumn than spring and summer, and 21-day-old drones produced more sperm than 14- and 35-day old drones. Drones from the four distinct lines were reared in separate colonies in the same apiary and given the same quality and quantity of supplemental feed. As a result, environmental effect or rearing in different colonies would have the largest effect followed by season effect on sperm numbers, genetics and age at sampling effect. Spermatozoa estimation is destructive and time consuming and will require careful management for environmental effects. However, selection for increased sperm production would be particularly helpful to increase reproductive competitiveness of selected stock in

breeding programs managed through uncontrolled open mating, as well as in artificial insemination given the limiting factor of sperm production and semen volume on the total number of possible queen inseminations. The cost-benefit of spermatozoa estimation must be carefully considered before further recommendation for inclusion in the wider queen breeding program.

### ***Semen volume***

Greater semen volume contributes to sperm competition as the frequency of a drone's worker offspring within a colony is strongly related to the drone's semen volume, with no significant effect of the insemination sequence of semen from different drones used to artificially inseminate queens (Schlüns, 2004). Greater worker subfamily frequency yields an increased likelihood a new queen will be reared from this subfamily (Kraus et al., 2003). Different genetic lines produce drones which vary in ejaculate volume and sperm numbers between them (Rhodes et al., 2011; Taha and Alqarni, 2013). Heritability of semen load volume is not known, and volume can be altered by seasonal and age effects in addition to genetics. Rhodes (2011) also found a genetic basis for semen release during manual drone eversion, which is a highly important consideration for breeding programs using artificial insemination.

Heritability studies should employ longitudinal measurement of drone semen volumes from individuals reared in different seasons from different lines within a closed breeding program that incorporates high semen volume into estimated breeding values for sires. Breeding programs employing artificial insemination should concurrently select for increased probability of semen release on manual eversion, as this is a large factor in wastage. Given Rhodes (2011) found a correlation of 0.54 between sperm number and semen volume, with little variation when age effect was added, selection for semen volume alone could serve as a proxy for sperm number selection, an expensive task from a labour perspective. Semen volume could be estimated within breeding programs utilising artificial insemination.

### ***Sperm viability***

Sperm viability is an expression of colony fitness, directly impacting on brood viability, the quality of queen mating and queen longevity. Unlike mammalian livestock, testing for honey bee sperm viability (i.e. – motility, morphology, mortality) is rare outside of research (Yániz et al., 2020) and destructive; therefore, visual assessment of brood viability is likely the most practical substitute for industry adoption.

### ***Miscellaneous traits***

Additional potential fertility traits such as drone genital morphology, sperm competition, age to sexual maturity and onset of senescence are either too destructive, costly or difficult to measure, negating any benefit of their inclusion in breeding programs. Age to sexual maturity may be a possible exception due to its importance in controlled mating programs or business diversification where drone semen is an output.

## Conclusion

Published studies on honey bee fertility traits of value in commercial enterprises focus on biotic and genetic factors affecting reproductive castes' expression of these traits with minimal focus on heritability or economic value. The minimal focus to date on heritability estimates and trait effect on profitability necessitates additional research into queen and drone weight, queen size and brood viability in order to determine their value in bee breeding programs. Once heritabilities are described, further research on the heterotic effects of specific crosses and the effect of intracolony interactions between subfamilies on colony fitness and production would benefit the ongoing viability of commercial enterprise by enabling more targeted selection.

## References

- Anderson, D (2004) Improving Queen Bee Production. Rural Industries Research and Development Corporation, Publication No. 04/153, Barton.
- Berg S, Koeniger N, Koeniger G, Fuchs S (1997) Body size and reproductive success of drones (*Apis mellifera* L.). *Apidologie* **28**, 449–460.
- Bieńkowska, M, Panasiuk, B, Węgrzynowicz, P, Gerula, D (2011) The effect of different thermal conditions on drone semen quality and number of spermatozoa entering the spermatheca of queen bee. *Journal of Apicultural Science* **55**, 161–168.
- Brascamp, EW, Bijma, P (2014) Methods to estimate breeding values in honey bees. *Genetic Selection Evolution* **46**, 1–15.
- Brascamp, EW, Willam, A, Boigenzahn, C, Bijma, P, Veerkamp, RF (2016) Heritabilities and genetic correlations for honey yield, gentleness, calmness and swarming behaviour in Austrian honey bees. *Apidologie* **47**, 739–748.
- Büchler, R, Berg, S, Le Conte, Y (2010) Breeding for resistance to Varroa destructor in Europe. *Apidologie* **41**, 393–408.
- Büchler, R, Andonov, S, Bienefeld, K, Costa, C, Hatjina, F, Kezic, N, Kryger, P, Spivak, M, Uzunov, A, Wilde, J (2013) Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research* **52**, 1–30.
- Cobey, SW, Tarpy, DR, Woyke, J (2013) Standard methods for instrumental insemination of *Apis mellifera* queens. *Journal of Apicultural Research* **52**, 1–18.
- Collins, AM (2000) Relationship between semen quality and performance of instrumentally inseminated honey bee queens. *Apidologie* **31**, 421–429.
- Collins, AM, Pettis, JS (2013) Correlation of queen size and spermathecal contents and effects of miticide exposure during development. *Apidologie* **44**, 351–356.
- Costa Maia, F, Lourenco, DAL, Tsuruta, S, Martins, E (2018) Selection criteria for improving honey production in Africanized honey bees. *Journal of Animal Science* **96**, 115.



- Danka, RG, Harris, JW, Villa, JD (2011) Expression of Varroa sensitive hygiene (VSH) in commercial VSH honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology* **104**, 745–749.
- De Souza, DA, Bezzerla-Laure, MAF, Francoy, TM, Gonçalves, LS (2013) Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence. *Genetics and Molecular Research* **12**, 5382-5391.
- Evans JP, Simmons, LW (2007) The genetic basis of traits regulating sperm competition and polyandry: can selection favour the evolution of good- and sexy sperm? *Genetica* **134**, 5-19.
- Evans, JD, Wheeler, DE (1999) Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 5575–5580.
- Evans, JD, Wheeler, D (2000) Expression profiles during honeybee caste determination. *Genome Biology* **2**, 1-6.
- Fisher II, A, Harrison, K, Love, C, Varner, D, Rangel, J (2018) Spatio-temporal variation in viability of spermatozoa of honey bee, *Apis mellifera*, drones in central Texas apiaries. *Southwestern Entomologist* **43**, 343–356.
- Hellmich, RL, Kulincevic, JM, Rothenbuhler, WC (1985) Selection for high and low pollen hoarding honey bees. *Journal of Heredity* **76**, 155–158.
- Hepperle, C, Hartfelder, K (2001) Differentially expressed regulatory genes in honey bee caste development. *Naturwissenschaften* **88**, 113–116.
- Hillesheim, E, Koeniger, N, Moritz, RFA (1989) Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. *Behavioral Ecology and Sociobiology* **24**, 291–296.
- Hoover, SER, Keeling, CI, Winston, ML, Slessor, KN (2003) The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**, 477-480.
- Hrassnigg, N, Crailsheim, K (2005) Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie* **36**, 255-277.
- Hunt, GJ, Guzman-Novoa, E, Fondrk, MK, Page Jr, RE (1998) Quantitative Trait Loci for honey bee stinging behavior and body size. *Genetics Society of America* **148**, 1203–1213.
- Jarolimек JP, Otis GW (2001) A comparison of fitness components in large and small honeybee drones. *American Bee Journal* **12**, 891–892.
- Jones, JC, Myerscough, MR, Graham, S and Oldroyd, BP (2004) Honey bee nest thermoregulation: diversity promotes stability. *Science* **305**, 402–404.
- Kahya, Y, Gençer, HV, Woyke, J (2008) Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. *Journal of Apicultural Research*, **47**, 118-125.
- Karasiński, JM (2018) The economic valuation of Australian managed and wild honey bee pollinators [Online]. Available at [www.weenbee.foundation.org.au/wp-content/uploads/2020/02/Karasiński-JM-2018-The-Economic-Valuation-of-Australian-Managed-and-Wild-Honey-Bee-Pollinators-in-2014-2015.pdf](http://www.weenbee.foundation.org.au/wp-content/uploads/2020/02/Karasiński-JM-2018-The-Economic-Valuation-of-Australian-Managed-and-Wild-Honey-Bee-Pollinators-in-2014-2015.pdf) (posted September 2018; verified 1 December 2020).

- Koeniger, G, Koeniger, N, Tingek, S, Phiancharoen, M (2005) Variance in spermatozoa number among *Apis dorsata* drones and among *Apis mellifera* drones. *Apidologie* **36**, 279-284.
- Kolmes, SA, Sam, Y (1991) Relationships between sizes of morphological features in worker honey bees (*Apis mellifera*). *Journal of the New York Entomological Society* **99**, 684-690.
- Kraus, FB, Neumann, P, Scharpenberg, H, Van Praagh, J, Moritz, RFA (2003) Male fitness of honeybee colonies (*Apis mellifera* L.). *Evolutionary Biology* **16**, 914-920.
- Kumar, NR, Kaur, N (2003) Seasonal influence on physico-chemical characteristics of honey bee semen. *Journal of Applied Zoological Research* **14**, 191-192.
- Laidlaw, HH, El-Banby, MA (1962) Inhibition of yellow body color in the honey bee: *Apis mellifera* L. *Journal of Heredity* **53**, 171-173.
- Laidlaw Jr, HH, Page Jr, RE (1997) 'Queen Rearing and Bee Breeding.' (Cheshire: Wicwas).
- Makert, GR, Paxton, RJ and Hartfelder, K (2006) Ovariole number—a predictor of differential reproductive success among worker subfamilies in queenless honeybee (*Apis mellifera* L.) colonies. *Behavioral Ecology and Sociobiology* **60**, 815-825.
- Mattila, HR and Seeley, TD (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**, 362-364.
- Mattila, HR, Burke, KM and Seeley, TD (2008) Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. *Proceedings of the Royal Society B* **275**, 809-816.
- Metz, BN, Tarpay DR (2019) Reproductive senescence in drones of the honey bee (*Apis mellifera*). *Insects* **10**, 1-17.
- Moritz RFA, Kryger P, Allsopp MH (1996) Competition for royalty in bees. *Nature* **384**, 31.
- Nelson, DL, Gary, NE (1983) Honey productivity of honeybee colonies in relation to body weight, attractiveness and fecundity of the queen. *Journal of Apicultural Research* **22**, 209-213.
- Oldroyd, BP, Fewell, JH (2007) Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology and Evolution* **22**, 408-413.
- Oldroyd, BP, Rinderer, TE, Harbo, JR and Buco, SM (1992) Effects of intracolony genetic diversity on honey bee (Hymenoptera: Apidae) colony performance. *Annals of the Entomological Society of America* **85**, 335-343.
- Oxley, PR, and Oldroyd, BP (2010) The Genetic Architecture of Honeybee Breeding. *Advances in Insect Physiology* **39**, 83-118.
- Page RE, Erickson EH (1988) Reproduction by worker honey bees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* **23**, 117-126.
- Page, RE, Fondrk, MK (1995) The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. *Behavioral Ecology and Sociobiology* **36**, 135-144.
- Page, RE, Kimsey, RB, Laidlaw Jr, HH (1983) Migration and dispersal of spermatozoa in spermathecae of queen honeybees (*Apis mellifera* L.). *Experientia* **40**, 182-184.



- Page, RE, Robinson, GE, Fondrk, MK (1995) Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* **36**, 387–396.
- Page, RE, Robinson GE (1994) Reproductive competition in queenless honeybee colonies (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* **35**, 99–107.
- Palmer, KA and Oldroyd, BP (2000) Evolution of multiple mating in the genus *Apis*. *Apidologie* **31**, 235–248.
- Rangel, J, Böröczky, K, Schal, C, Tarry, DR (2016) Honey bee (*Apis mellifera*) queen reproductive potential affects queen mandibular gland pheromone composition and worker retinue response. *PLoS ONE* **11**, 1-16.
- Rangel, J, Fisher, A (2019) Factors affecting the reproductive health of honey bee (*Apis mellifera*) drones—a review. *Apidologie* **50**, 759–778.
- Rhodes, JW (2011) 'Quality of commercially reared queen and drone honey bees (*Apis mellifera* L.) in eastern Australia.' PhD thesis, The University of Western Sydney, Australia.
- Rinderer, TE, Collins, AM, Pesante, D (1985) A comparison of Africanized and European drones: weights, mucus gland and seminal vesicle weights, and counts of spermatozoa. *Apidologie* **16**, 407-412.
- Rousseau, A, Fournier, V, Giovenazzo, P (2015) *Apis mellifera* (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of breeding. *Canadian Entomologist* **147**, 702–711.
- Schlüns, H, Schlüns, EA, van Praagh, J, Moritz, RFA (2003) Sperm numbers in drone honeybees (*Apis mellifera*) depend on body size. *Apidologie* **34**, 577-584.
- Schlüns, H, Koeniger, G, Koeniger, N, Moritz, RFA (2004) Sperm utilization pattern in the honeybee (*Apis mellifera*). *Behavioral Ecology and Sociobiology* **56**, 458–463.
- Seeley, TD and Tarry, DR (2007) Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society B* **274**, 67–72.
- Soller, M, Bar-Cohen, R (1967) Some observations on the heritability and genetic correlation between honey production and brood area in the honeybee. *Journal of Apicultural Research* **6**, 37-43.
- Spivak, M and Reuter, GS (1998) Performance of hygienic honey bee colonies in a commercial apiary. *Apidologie* **29**, 291-302.
- Spivak, M and Reuter, GS (2001) Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behaviour. *Apidologie* **32**, 555-565.
- Stürup M, Baer-Imhoof B, Nash DR, Boomsma JJ, and Baer B (2013) When every sperm counts: factors affecting male fertility in the honeybee *Apis mellifera*. *Behavioral Ecology* **24**, 1192–1198.
- Taha, EKA, Alqarni, AS (2013) Morphometric and reproductive organs characters of *Apis mellifera jemenitica* drones in comparison to *Apis mellifera carnica*. *International Journal of Scientific & Engineering Research* **4**, 411-415.
- Tarry D, Keller J, Caren J, Delaney D (2011) Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. *Insectes Sociaux* **58**, 569–574.

Woyke, J (1960) Natural and instrumental insemination of queen bees. *Pszczelnicze Zeszyty Naukowe* **4**, 183–275.

Woyke, J (1971) Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *Journal of Apicultural Research* **10**, 45-55.

Woyke, J and Jasinski, Z (1973) Influence of external conditions on the number of spermatozoa entering the spermatheca of instrumentally inseminated honeybee queens. *Journal of Apicultural Research* **12**, 145-149.

Yániz, JL, Silvestre, MA, Santolaria, P (2020) Sperm quality assessment in honey bee drones. *Biology* **9**, 1-16.

Zaitoun S, Al-Majeed Al-Ghzawi A, Kridli R (2009) Monthly changes in various drone characteristics of *Apis mellifera ligustica* and *Apis mellifera syriaca*. *Journal of Entomological Science* **12**, 208–214.