



Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence

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ABSTRACT. There has been much speculation about which phenotypic traits serve as reliable indicators of productivity in queen honeybees (*Apis mellifera*). To investigate the predictive value of queen body weight on colony development and quality, we compared colonies in which queens weighed less than 180 mg to those in which queens weighed more than 200 mg. Both groups contained naturally mated and instrumentally inseminated queens. Colonies were evaluated on the basis of performance quality, growth rate, and queen longevity. We found that queen body weight was significantly correlated with fecundity and colony quality. Heavy queens exhibited the most favorable performance and colony quality. In contrast, naturally mated,

with the opposite trend being obtained for light-weight queens. We found no statistically significant difference between instrumentally inseminated queens and naturally mated queens. Our results support the use of queen body weight as a reliable visual (physiological) indicator of potential colony productivity in honey bees to enhance genetic lines in genetic improvement programs.

Key words: *Apis mellifera*; Queen; Body weight; Longevity; Reproduction

Once the reproductive system of a queen *Apis mellifera* honeybee is fully developed, the individual becomes the progenitor of all subsequent colony members. In addition to continuing her genetic line, the production of pheromones by the queen bee directly influences the organization colony functions. The queen's pheromones also regulate the homeostatic conditions within the colony through (Butler, 1960; Bienefeld and Pirchner, 1990). Furthermore, the quantity and quality of eggs being laid is also essential. Boch and Jamieson (1960) observed that the body weight of queens at emergence is positively and significantly correlated with the brood area. The amount of open brood is important because it leads to a strong adult population, and stimulate increased foraging behaviors among adult bees. This connection between open brood and foraging is supported by observations that pheromones produced in the mandibular glands of larvae prompt the adults to forage for pollen (Pankiw, 1998). Subsequent increases in pollen quantity, in turn, are believed to be distributed throughout the colony's biomass. As a result, nursing bees provide an increased food supply to the queen, stimulating a higher rate of posture and brood production, which enhance population growth (Pankiw and Page, 2001; Le Contel et al., 2001; Pankiw, 1998, 2004).

Anatomically, the queen has 2 ovaries, each of which contains 180-200 ovarioles (Snodgrass, 1956). The reproductive life of the queen begins after the ovarioles mature, which is approximately 7 days into adult life; however, the full development of a queen's ovaries only occurs after mating. At this point, vitellogenesis begins, oocytes mature, and egg laying is initiated (Patricio and Cruz-Landim, 2002; Tanaka and Hartfelder, 2004). A queen's potential for ovariole production has been shown to correspond positively with her weight (Hoopingarner and Farrar, 1959).

The queen mates with several drones. Subsequently, the drone semen (containing sperm) enters through the vaginal orifice into the oviduct, and subsequently migrates to the spermatheca. Once the semen enters the spermatheca, it is sustained throughout the fertile life of the queen and gradually used for the fertilization of oocytes. Subsequently, no further mating occurs; hence, sperm reserves are constrained by the capacity of a queen's spermatheca. As a queen's semen reserves near depletion, the colony begins the process of selecting a replacement queen (Winston, 1979). Thus, the ability of a queen to store sperm has direct implications on her productivity and longevity.

Therefore, the body weight of the queen honeybee might serve as a potential indicator of colony productivity. The diameter and volume of a queen's spermatheca is believed to be positively correlated with her body weight at emergence; hence, a larger queen is expected to have a greater ability to store sperm (Woyke, 1967, 1971; Corbella and Gonçalves, 1982; Medina, 1993; Kahya et al., 2008; Delaney et al., 2010). Since a queen's body weight is linked

to the anatomical development of the reproductive organs, this characteristic might prove a reliable indicator of reproductive quality and queen longevity. However, reports remain limited about the actual influence of this characteristic on colonial development and productivity, which is important in the context of genetics improvement programs. Therefore, the present work investigates the significance of a queen's body weight at emergence to her fertility and longevity, in addition to the development and productivity of resultant colonies.

MATERIAL AND METHODS

Queens and colonies

Queens were reared following standard queen rearing procedures (Laidlaw and Page, 1997), using 1-3 day old larvae. Two days prior to emergence, individual royal cells were transferred to an incubator, and were weighed immediately after emerging. To minimize genotypic variation, all queens were produced using larvae from the same colony source.

The experiment contained 4 experimental groups: 1) naturally mated queens with a body weight of <180 mg (low line = L) (NML); 2) instrumentally inseminated queens with a body weight of <180 mg (IIL); 3) naturally mated queens with a body weight of >200 mg (high line = H) (NMH); and 4) instrumentally inseminated queens with a body weight of >200 mg (IIH). Three females were used in each group, producing a total of 12 observed colonies.

Each queen was marked with an individual identification label, and introduced to nucleus colonies (nucs) of similar biomass (tree combs of brood covered workers). Naturally mated queens were first introduced to vertical observation hive colonies, to collect data about mating behavior. At the beginning of egg laying, the queens were relocated into the nucs. Instrumentally inseminated queens were weighed, marked, and introduced to nucs until they reached reproductive maturity (7 days after emergence). At this point, they were instrumentally inseminated with 6 μ L semen and returned to the hive where they had been initially introduced. Both light and heavy weight queens received the same treatment (e.g., semen quantity) for the instrumentally inseminated treatment.

Colony performance

Our observations of colony development began 20 days after the queens began laying eggs. We inspected the development of all colonies by mapping the area of the combs every 15 days (Medina, 1993) over a 1-year period (2008-2009). The area of the comb was measured following five classifications; whereby, the area occupied by eggs, open and closed broods, and pollen stocks was estimated as 0, 25, 50, 75, or 100%. We conducted weekly field-surveys of each colony to check for the presence of the queen, estimate her longevity, and record the rate of each colony's population growth. Once the bee populations reached a certain size, the colonies were transferred to larger hives, and the time of this expansion was documented.

Statistical analysis

Data from the 4 experimental groups were compared statistically by the Student *t*-test and ANOVA using the Sigma Stat for Windows version 3.5 software.

RESULTS

Onset of egg laying

The NMH and NML queens began laying eggs when they reached 9.88 ± 3.02 and 7.77 ± 1.86 days old, respectively, whereas the IIH and IIL queens started laying eggs slightly later, at 10.62 ± 0.51 and 10.25 ± 0.46 days, respectively. These differences were not statistically significant ($P = 0.079$ and $P = 0.250$, respectively, Figure 1A and B). However, comparisons between NM and II queens show that naturally mated queens began laying eggs earlier compared to instrumentally inseminated queens, at 8.70 ± 2.68 and 10.47 ± 0.51 days old, respectively ($P = 0.011$, Figure 1C).

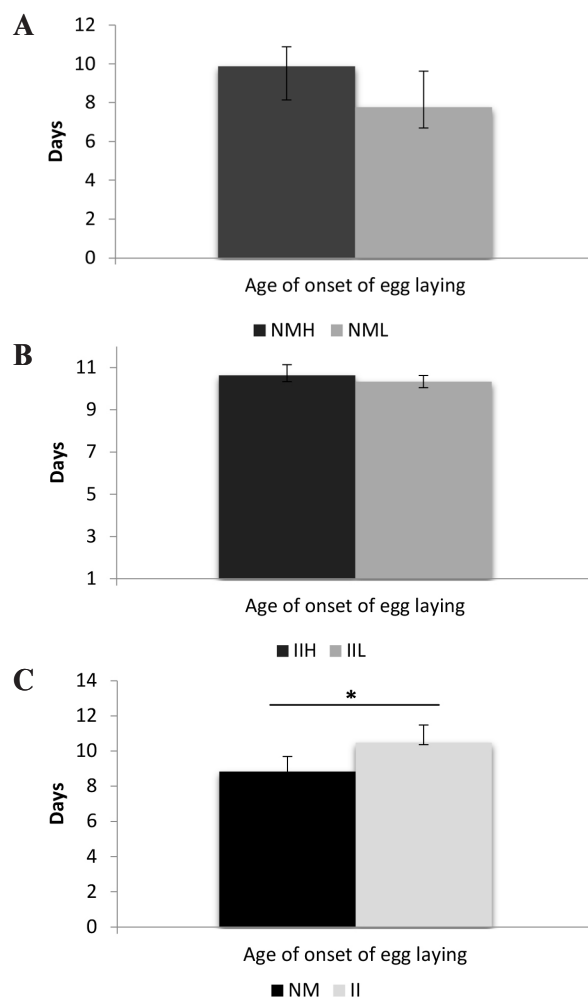


Figure 1. Egg laying onset in days of Africanized honey bee queen's groups (*Apis mellifera*): **A.** average of naturally mated heavy queens (NMH) and naturally mated light queens (NML); **B.** average of instrumentally inseminated heavy queens (IIH) and instrumentally inseminated light queens (IIL); **C.** average of natural mated queens (NM) and instrumentally inseminated queens (II). *Differences statistically significant at $P < 0.05$.

A statistically significant difference was only obtained for the closed brood area of heavy versus light naturally mated queens ($P = 0.003$). For heavy versus light, naturally mated queens, there was no statistical difference in the relative of colony space for egg-laying area ($P = 0.117$), open brood area ($P = 0.483$), or stored pollen area ($P = 0.407$). There was only a statistically significant difference for the open brood area of heavy and light instrumentally inseminated queens ($P = 0.001$). There was no significant difference in the relative to egg laying area ($P = 0.498$), closed brood area ($P = 0.737$), or stored pollen ($P = 0.307$) (Figure 2).

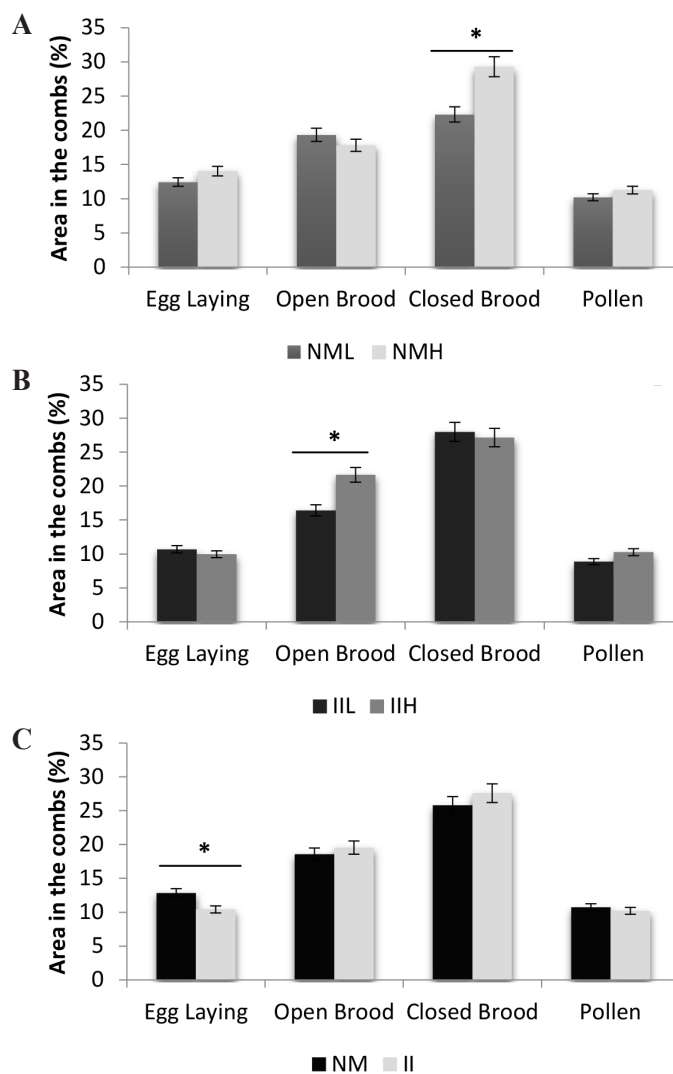


Figure 2. Composition of the total area of the combs (in %) of Africanized honey bee queen colonies (*Apis mellifera*): egg laying, open brood, closed brood, and pollen storage. **A.** Average of heavy and light queens naturally mated (NML and NMH, respectively); **B.** average of heavy and light queens instrumentally inseminated (IIH and IIL, respectively); **C.** average of naturally mated queens and instrumentally inseminated queens (NM and II, respectively). *Differences statistically significant at $P < 0.05$.

Comparisons of the colonies produced by naturally vs. instrumentally inseminated queens only yielded a statistically significant difference for the relative area of the combs devoted to egg laying. In contrast, non-significant differences were observed for the open brood area ($P = 0.828$), closed brood area ($P = 0.420$), and stored pollen area ($P = 0.397$) (Figure 2).

Colony expansion time

The rate at which colonies grew differed among the 4 experimental groups; hence, each colony was transferred to larger hives at different times. The NML group exhibited the lowest expansion rate, with none of the colonies in this group being transferred to larger hives. In comparison, the IIH group exhibited the fastest growth rate (average of 140 ± 46.18 days), while the NMH and IIL groups produced similar results (average of 117 ± 21.70 days and 116 ± 18.73 days, respectively) (Table 1). In other words, an average of 24 days separated the rate at which colonies from light queens vs heavy queens reached maximum colony capacity. Heavier queens generated faster population growth compared to light queens. Increased disparities in expansion rates generally corresponded with the high-flow pollen season, during spring.

Table 1. Colony performance (expansion time) and queen longevity of four groups of Africanized honey bee queens naturally mated (NML and NMH) and instrumentally inseminated (IIL and IIH).

		Weight (mg)	Colony expansion time (days)	Longevity (days)
Naturally mated	Light	160.0	X	279
		174.0	X	336
		170.0	X	285
	Average	168.0	X	300.00 ± 31.32
	Heavy	210.0	92	425
		215.0	131	605
		225.0	128	760
Average		216.7	117 ± 21.7	596.66 ± 167.65
Instrumentally inseminated	Light	176.0	140	186
		152.0	140	286
		165.0	220	372
		Average	164.3	140 ± 46.18
	Heavy	203.0	131	375
		209.0	122	217
		203.0	95	329
Average	205.0	116 ± 18.73	307.00 ± 81.26	

L = light queens, H = heavy queens. X = colonies that had no sufficient population growth to transfer to a larger cage.

Longevity of queens

The mean longevity of NML and NMH queens was 300.00 ± 31.32 days (around 9 months), and 596.66 ± 167.65 days (around 19 months), respectively. The mean longevity for IIL and IIH queens was 281.33 ± 93.08 days and 307.00 ± 81.26 days (around 9 months), respectively. Although the 10-month difference in longevity between NML and NMH queens was statistically significant ($P = 0.045$), the 26-day difference in average longevity between IIL and IIH queens ($P = 0.391$) was not (Table 1). Nonetheless, comparisons between the mean longevity of naturally mated heavy queens and the other groups did generate statistically significant differences ($P = 0.001$).

DISCUSSION

We found that significant variation in the performance of the colonies was correlated with body weight differences between honeybee queens, regardless of whether fertilization was natural or instrumental. Although some comparisons were not statistically significant, noteworthy biological significance was demonstrated. Differences in the developmental time of NML vs NMH queens might have been caused by discrepancies in the rate at which egg laying began. In general, light queens develop from older larvae that are at an advanced development stage (around the 3rd instar), whereas heavy queens typically develop from newly hatched eggs (Dedej et al., 1998). When colonies lose their old queen, workers begin the production of new queens using larvae that vary in age and development (Visscher, 1993). The first queens to emerge often attempt to eliminate other potential queens before they emerge from their royal cells as rivals. This evolutionary strategy might result in early-emerging queens becoming the new queen of the colony. Because these early-emerging queens are often light-weight, selection is generally believed to favor light-weight queens (DeGrandi-Hoffman et al., 1998).

We observed some differences between light and heavy queen experimental groups regarding colony performance and development, particularly with respect to the amount of area designated to closed broods. The greater area designated to closed broods was positively correlated to the fast rate of population expansion and growth among the colonies of heavy queens.

The capacity for brood production is one of the main factors that regulates whether colony expansion and reproduction generates highly populated colonies. This factor could be used to predict high production rates, which is of interest to genetic improvement programs. This success in brood production is attributed to the colony's efficiency in converting food to biomass, in addition to the viability of offspring (Page, 1980). Heavy queens improve the size of the brood area and, thus, the rate of population expansion, with heavy queen colonies being transferred to larger hives about 24 days earlier compared to light queen colonies in the current study. This finding is relevant for beekeepers seeking to maximize productivity during the pollination season.

Because all 4 experimental groups developed under identical environmental conditions, we believe that divergences in colony performance are mainly caused by intra-colonial factors, particularly those derived from the phenotypic distinctions between the groups. For example, the influence of queens on their colonies is known to be manifested in at least 3 ways, including: the genotype she passes on to her offspring, the quantity and quality of her offspring, and the pervasive dispersal of her pheromones (Bienefeld and Pirchner, 1990; Bienefeld et al., 2007). Queen pheromones are generally considered important in the regulation of foraging behavior among worker populations (Pankiw, 1998; Pankiw et al., 2004).

Increased foraging among workers is believed to improve a colony's viability during periods of food scarcity, and is therefore relevant to the development of a robust and populated colony (Page, 1980). Although the open brood area differed minimally among the 4 experimental groups, the closed brood area differed by around 7% between NML and NMH, which significantly influenced the proper development of the colony.

Meanwhile, another potential difference between heavy and light queens is the degree of genetic diversity in the colonies of each respective queen-type. This hypothesis links to the stated differences in the diameter and volume of the spermatheca; whereby, heavy queens are larger and able to store more sperm, and hence mate with a greater number of drones compared to light-weight queens. Consequently, heavy queens should produce more robust colonies,

with greater genetic variation (Woyke, 1967; Tarpy and Page, 2000, Kraus et al., 2005; Kahya et al., 2008; Tarpy et al., 2011). This increase in diversity should produce a more robust colony that is able to survive a broader range of environmental pressures (Oldroyd and Fewell, 2007).

The only significant difference between colonies produced from naturally mated and instrumentally inseminated queens was found for egg laying. However, compared to the other characteristics (such as the rate of colony expansion), this difference was not a determining factor. Supporting previous studies, we found that instrumental insemination does not significantly impact a queen's function within the colony (Woyke, 1971; Cobey, 1998; Pritsch and Bienefeld, 2002; Abdulaziz et al., 2003).

Despite these negligible outcomes between natural and instrumental insemination methods, greater pollen stores were observed in colonies with naturally fertilized queens. This inconsistency was considered by Matilla et al. (2008), who observed that colonies featuring high genetic variability also exhibit increased foraging behavior in colonies headed by monogamous queens. According to the authors' argument, increased genetic variability is positively associated with foraging behavior. In other words, more genetically diverse colonies explore larger areas of food sources compared to monogamous colonies, which is a beneficial adaptation for survival in adverse natural conditions. Hence, this increased genetic variation within a population corresponds to an increased probability for developing an adaptive response to a given environment (Myerscough and Oldroyd, 2004; Matilla et al., 2008; Matilla and Seeley, 2007, 2011).

The longevity of instrumentally inseminated queens was comparable for both light and heavy queen populations following insemination. In contrast, heavier queens lived about 10 months longer than light queens in the naturally mated, or in the inseminated groups. This result represents a huge difference in productivity for beekeeping activities. This longer longevity has several benefits including avoiding the loss of the queen in the middle of the highly productive season, which could cause a break in the stocking of food. This increased longevity might be attributed to the effect of larger semen stock capacities in heavy queens (Woyke, 1967; Medina, 1993; Kahya et al., 2008). Alternatively, other physiological differences might also contribute to this difference; hence, further investigation about what regulates queen longevity is required.

Considering the overall performance and longevity of colonies with heavy queens, we recommend the continued use of this feature as a phenotypic indicator of higher productivity and better economic benefits in honeybees (*A. mellifera*). However, further research is required focusing on the implications of queen body weight in relation to various physiological characteristics, and how these phenotypes potentially influence brood quality and colony performance, to identify the most relevant characteristics.

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