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Original Article

THE EFFECTS OF STARVATION OF HONEY BEE LARVAE ON REPRODUCTIVE QUALITY AND WING ASYMMETRY OF HONEY BEE DRONES

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Summary

Starvation during larval development has a negative effect on adult worker honey bees (*Apis mellifera* L.), but much less is known about the quality of drones starved during their development. We verified how starvation on the second day (early starvation) or the sixth day (late starvation) of larval development affects body mass, ejaculated semen volume and forewing size, shape, size asymmetry and shape asymmetry in drones after emergence. The larvae were starved for ten hours by being separated from nursing bees with a wire mash for 10 hours either early or late during larval development. Drones starved both early and late were smaller (254.1 ± 1.97 mg and 239.4 ± 2.12 mg, respectively) than the control regularly fed individuals (260.9 ± 2.01 mg), and their wing size changed as well (control: 889.76 ± 1.06; early: 880.9 ± 1.17; late: 868.05 ± 1.48). Starvation at a later phase of larval development caused more pronounced effects than at an earlier phase. On the other hand, ejaculated semen volume (control: 0.7 ± 0.043 µl; early: 0.88 ± 0.040 µl; late: 1.08 ± 0.031 µl), wing size asymmetry (control: 0.49 ± 0.025; early: 0.51 ± 0.026; late: 0.52 ± 0.03) and wing shape asymmetry (control: 17.4 ± 0.47 x 10-3; early: 16.9 ± 0.41 x 10-3; late: 17.6 ± 0.43 x 10-3) were not affected by starvation. This suggests that drones attempt to preserve characters which are important for their future reproduction.

Keywords: ***Apis mellifera,*** **drone body mass, semen volume, starvation, wing asymmetry**

INTRODUCTION

Honey bees (*Apis mellifera* Linnaeus, 1758) require significant and regular food intake for optimal growth during development. Royal jelly and partially pollen are the main sources of proteins, lipids, vitamins and minerals, and nectar is the main source of carbohydrates and water (for review see: Haydak, 1970; Babendreier et al., 2004; Brodschneider & Crailsheim, 2010). The quality of royal jelly depends on the quality, quantity and nourishment of nursing bees (Haydak, 1970; Brodschneider & Crailsheim, 2010). Under natural conditions, food (nectar and pollen) quality and its availability for bees change seasonally because it depends on the diversity and abundance of flowering food plants (Köppler et al., 2007; Odoux et al., 2012). Artificially induced changes in food availability and/or feeding regimes of worker larvae have been shown to affect gene transcription (Corby-Harris et al., 2014), immunocompetence (Alaux et al., 2010), larval development, longevity (Mattila & Otis, 2006; Scofield & Mattila, 2015), body size and ovary development level (Hoover et al., 2006; Wang et al., 2014) and hypophyryngeal gland development (Pernal & Currie, 2000). The latter was proved to be especially sensitive to changes in feeding in the 5th instar larvae (Wang et al., 2014).

In extreme conditions, workers were found to cannibalize young larvae to feed older ones (Schmickl & Crailsheim, 2001; 2002) or give up brood rearing completely - usually starting with the drone larvae, to avoid producing highly impaired sexual individuals (Kunert & Crailsheim, 1985; Crailsheim & Hrassnigg, 1998; Imdorf et al., 1998). For optimal development, drones require significant and regular food intake whose frequency depends on the larvae's age (Haydak, 1970, Huang & Otis, 1991; Boes, 2010). Workers on the other hand adjust the number of produced drones to the environmental conditions including colony size and food availability (Boes, 2010). Despite chronic reduction, but not complete elimination, of available fresh pollen in colonies, nurse bees were able to a certain degree to still rear drones (Czekońska et al., 2015). However, drones reared during such long-term limitation of pollen were smaller and ejaculated less semen but the number and viability of spermatozoa in the ejaculate was not affected by it (Czekońska et al., 2015).

No information is available on how acute starvation will affect the quality of different aged drones. Rarely observed in natural conditions, acute starvation's influence on larval development can help to understand larval nutrition requirements. Studies on worker development cannot be used to predict drone development because drones have different protein and carbohydrate requirements as larvae than workers (Hrassnigg & Crailsheim, 2005). Plus drones' reproductive quality can differ not only due to differences in their body size (Berg et al., 1997; Couvillon et al., 2010), which is mainly affected by the size of cells they were reared in (Berg, 1991; Berg et al., 1997; Schlüns et al., 2003), but also depend on environmental and genetic factors (Gençer & Firatli, 2005; Zaitoun et al., 2009; Taha & Alqarni, 2013).

Nutritional shortage can also affect the developmental stability of offspring and consequently their body symmetry (Ohlsson & Smith, 2001; Grønkjær & Sand, 2003). Asymmetry of wing morphology in drones was found to be correlated with their quality by Jaffé & Moritz (2010). Honey bees are among the most intensively studied insect species and there is a significant amount of literature concerning their general biology. Yet, little is known about their symmetry measures and often the results of these studies are inconsistent. Some studies have failed to demonstrate detectable changes in the level of fluctuating asymmetry (Clarke, Brand, & Whitten, 1986; Smith, et al., 1997; Jones et al., 2005) while others did (Brückner, 1976, Ondo Zue Abaga et al., 2011). However, Jaffé & Moritz (2010) found that drones captured in drone congregation areas have more symmetrical wings than drones found in their maternal colony, suggesting that less asymmetrical individuals are of higher reproductive quality.

The aim of our study was to describe how acute starvation at the beginning (2nd day of larval development) and at the end of the larval feeding period (6th day of larval development) affects drone quality measured as body mass at emergence, amount of ejaculated semen and forewing asymmetry.

MATERIAL AND METHODS

Experimental setup

Drones were reared in their natal colonies throughout the experiment. All colonies were healthy with only low levels of mite infection reaching not more than 4.4 infected individuals emerging/100 drones/colony. The experiment was performed in May with two hives used at the beginning and two at the end. One of the hives from the end of the month did not produce enough drones and was excluded from the experiment. The remaining colonies were named "A", "B", and "C" and each housed in a Wielkopolski hive with a two-year old queen *A. mellifera carnica*, provided by a queen breeder, and roughly 30,000 workers.

The hive consisted of two deep hive bodies with ten combs (360 × 260 mm) each and separated by a queen excluder. Five or six worker combs filled with brood in all developmental stages and four combs containing food were located in each hive body and one experimental drone comb were placed in the upper hive. On day one, each colony's queen was isolated in a frame cage on a new drone comb which comb was divided into three separate sectors, 1/4 + 1/4 +1/2, all available for the queen to lay eggs. After the first 24 hours, the queen was then placed in the lower hive body, while the experimental comb with freshly laid eggs was placed above among the other combs with developing larvae. The queen excluder placed between the hive bodies prevented the queen from further laying on the experimental comb. In each colony, drones were either raised undisturbed and fed continuously by the workers (control) or starved during the 2nd (early starvation) or 6th (late starvation) day of larval development for ten hours in each treatment. Starvation was achieved by covering 1/4 of the drone comb with metal wire mesh with 3 mm holes. The mesh was placed at least 0.5 cm from the edge of the drone cells to separate them physically from nursing bees and to avoid having the starved larvae crawl out of the comb.

One day prior to the expected emergence of drones, the combs were moved to incubators with a constant temperature of 34.5°C. During the next few days, the incubators were inspected every two hours. The first 30 individuals emerging from each colony were anaesthetized using CO_2 (Human et al., 2013) and their total body mass was determined using a RADWAG PS 210/C/2 analytical balance with a readability of 1 mg. The forewings of each measured bee were dissected for further analysis. The rest of the drones emerged over the next 24 hours and were moved to their maternal hives and stored in a cage made of queen excluder until semen collection. The semen was collected from drones when they were at the age of 15 days. At this age drones are mature and ready for mating (Cobey et al., 2013).

From each colony, three groups each of thirty individuals were sacrificed for semen and forewings to be collected. The thorax was pressed to evert the endophallus (Czekońska & Chuda-Mickiewicz, 2015) from which semen was collected with a glass capillary of pre-determined volume, diameter and length to assess the total amount of ejaculated semen. Forewings from individuals that did not survive until semen collection were also collected throughout the experiment. The age at which wings are collected does not affect their asymmetry, as it is determined during wing development of the larva and does not change during the life span of adult drones.

Wing measurements

Wings were mounted in photographic frames and scanned using a Nikon Super CoolScan 5000 ED scanner (resolution 2400 dpi). Nineteen landmarks (Fig. 1a, b, c) were determined on the forewings with the DrawWing software (Tofilski, 2004). Each wing was measured three times independently of one another and used to assess measurement error (Palmer, 1994; Graham et al., 2010), which was found to be relatively small in all individuals. Individuals with destroyed or dirty wings were excluded from further analysis. A total of 629 drones (220 from the control group, 223 from the early-starvation group and 186 from the late-starvation group) were measured.

The configurations of landmarks were aligned using Procrustes superimposition (Dryden & Mardia, 1998) in MorphoJ software (Klingenberg, 2011). The centroid size was used for measuring forewing size, (Dryden & Mardia, 1998). Wing size asymmetry was measured as the difference between the centroid size of the right and the left wing divided by the mean centroid size and multiplied by 100 (percentage of centroid size difference between left and right wing), while shape asymmetry was measured as the Procrustes distance between the shape of the right and the left wing called Procrustes FA score.

Statistical analysis

Total body mass and semen amount were analyzed using two-way ANOVAs with colonies A, B and C and the experimental group (control, early starvation, and late starvation) as factors. Wing size was correlated to body mass using Pearson's correlation coefficient.

Directional asymmetry and fluctuating asymmetry were analyzed. Directional asymmetry of wing size was analyzed by comparing the centroid size of the right and the left wings using t-test for paired comparison. On the other hand, directional asymmetry of wing shape was analyzed by comparing the Procrustes coordinates of the right and the left wing using one-way MANOVA. Wing size was compared between experimental groups and colonies using two-way ANOVA. Wing shape was

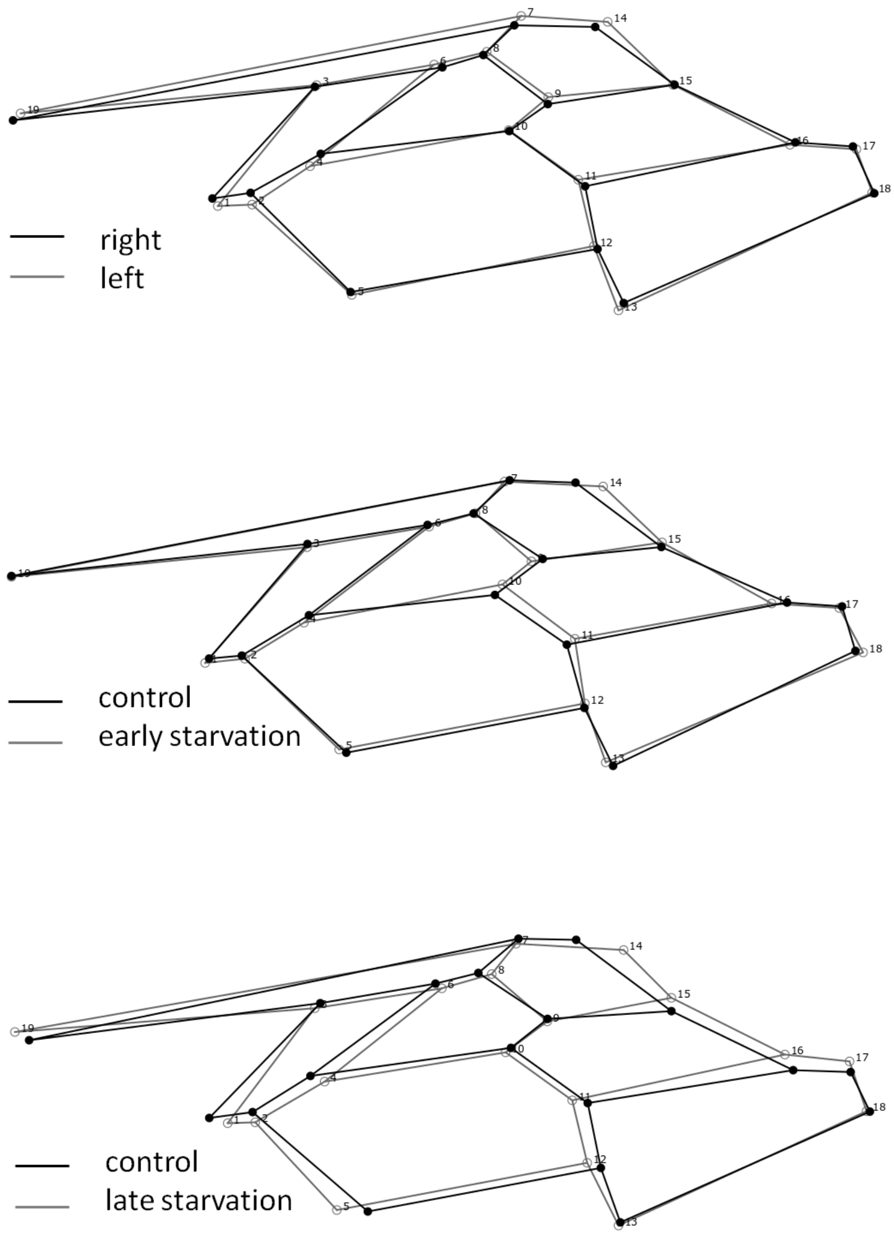

Fig. 1. Differences in shape of honey bee drone forewings. The lines represent wing veins and dots represent 19 landmarks used for wing measurements. a) scheme of forewing venation of the right and left wing; b) scheme of forewing venation of regularly fed (control) drones and drones starved during their 2nd day of larval development (early starvation); c) scheme of forewing venation of regularly fed (control) drones and drones starved during their 6th day of larval development (late starvation). Differences in shape were enlarged fifteen times to make them more visible.

compared between experimental groups and colonies using MANOVA based on Procrustes coordinates. Fluctuating asymmetry of wing size was analyzed by comparison of the absolute values of centroid size difference between the left and the right wing using two-way ANOVA. Wing shape asymmetry was compared using two-way ANOVA based on Procrustes FA score. All measures analyzed with ANOVA fulfilled the assumptions of analysis of variance and were followed by Tukey's test for uneven sample sizes for comparison between experimental groups and colonies. All analyses were performed using Statistica software v.10 (StatSoft Inc. 2011).

RESULTS

The body mass of drones was significantly affected by starvation ($F(2, 261) = 45.89$, $p < 0.001$). Starvation during early and late larval

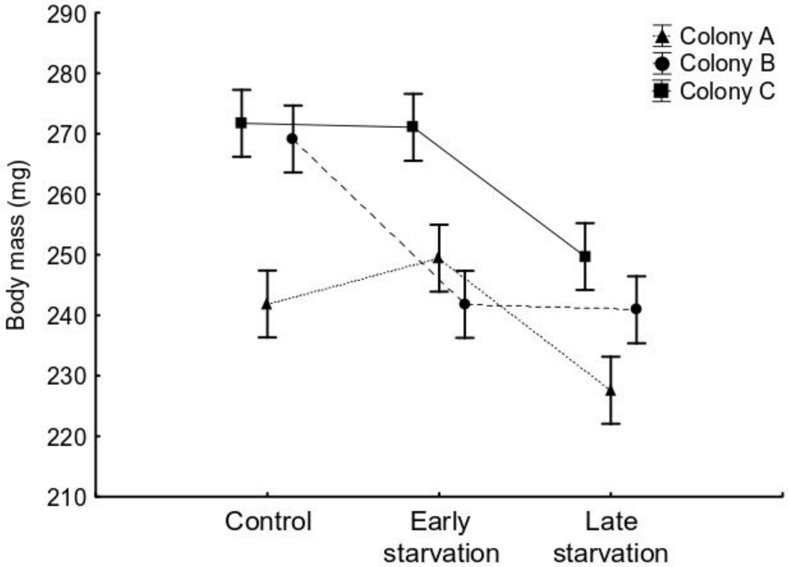

Fig. 2. Body mass (± SE) at emergence of drones originating from three unrelated colonies regularly fed (control) or starved for ten hours during the 2nd (early starvation) or the 6th-day (late starvation) of larval development.

development caused a decrease in body mass compared to control conditions amounting to 2.6% and 8.2%, respectively (Tab. 1a). Drone body mass also differed between colonies ($F(2, 261) = 57.36$, $p < 0.001$) (Tab. 1b). Additionally, a significant interaction between the treatment groups and colonies was revealed ($F(4, 261) = 11.07$, $p < 0.001$) (Fig. 2). The semen volume obtained from drones was not affected by starvation ($F(2, 149) = 2.48$, $p = 0.088$) (Tab. 1a) but significantly differed among colonies ($F(2, 149) = 25.25$, $p < 0.001$) (Tab. 1b) and with no interaction between treatment groups and colonies ($F(4, 149) = 2.0$, $p = 0.094$).

Wing size was significantly and positively correlated to body mass ($r = 0.54$, $p < 0.001$).

We found a clear directional asymmetry of both wing size and wing shape. Right wings ($t(1, 629) = - 6.4$, $p < 0.001$) in drones were significantly larger (880.9 ± 20.02) than left (879.5 ± 19.95) and wing shape differed significantly between them ($F(34, 1223) = 4.82$, $p < 0.001$).

Wing size differed between both the experimental groups ($F(2, 620) = 91.1$, $p < 0.001$) (Tab. 1a)

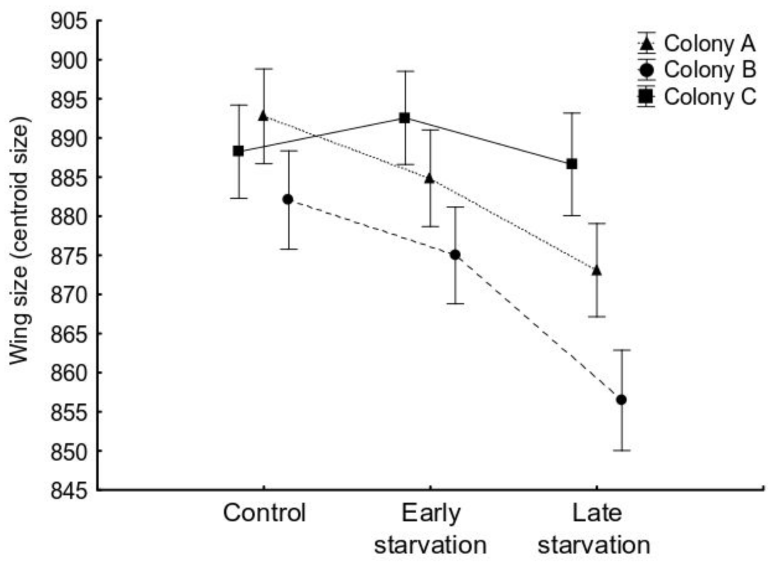

Fig. 3. Wing size (± SE) of drones originating from three unrelated colonies regularly fed (control) or starved for ten hours during the 2nd (early starvation) or the 6th-day (late starvation) of larval development.

Table 1
Body mass, amount of ejaculated semen, wing size, wing size asymmetry, and Procrustes FA score (± SE) of drones regularly fed (control) or starved for ten hours during the 2nd (early starvation) or the 6th-day (late starvation) of larval development (**a**) and originating from three unrelated colonies (**b**)

a)

Measure	Control	Early starvation	Late starvation
Body mass (mg)	260.9 ± 2.01[a]	254.1 ± 1.97[b]	239.4 ± 2.12[c]
Amount of semen (μl)	0.85 ± 0.041	0.94 ± 0.04	0.99 ± 0.046
Wing size	889.76 ± 1.06[a]	880.9 ± 1.17[a]	868.05 ± 1.48[b]
Wing size asymmetry	0.49 ± 0.025	0.51 ± 0.026	0.52 ± 0.03
Procrustes FA score	$17.4 \pm 0.47 \times 10^{-3}$	$16.9 \pm 0.41 \times 10^{-3}$	$17.6 \pm 0.43 \times 10^{-3}$

b)

Measure	Colony A	Colony B	Colony C
Body mass (mg)	239.6 ± 2.15[a]	250.6 ± 2.07[b]	264.2 ± 1.69[c]
Amount of semen (μl)	0.70 ± 0.04[a]	0.88 ± 0.04[b]	1.08 ± 0.03[c]
Wing size	882.9 ± 1.22[a]	874.65 ± 1.52[b]	883.3 ± 1.21[a]
Wing size asymmetry	0.56 ± 0.028[a]	0.51 ± 0.027[ab]	0.45 ± 0.024[b]
Procrustes FA score	$17.5 \pm 0.41 \times 10^{-3}$	$17.5 \pm 0.43 \times 10^{-3}$	$17.0 \pm 0.48 \times 10^{-3}$

Different letters (a, b, c) indicate statistically significant difference among groups.

and the colonies ($F(2, 620) = 27.6$, $p < 0.001$) (Tab. 1b). These factors also interacted ($F(4, 620) = 10.0$, $p < 0.001$) (Fig. 3).Wing size asymmetry only differed among colonies ($F(2, 620) = 4.6$, $p = 0.010$) (Tab. 1b) but not between treatments (($F(2, 620) = 0.5$, $p = 0.640$) (Tab.1a), and no interaction between colony and treatment was found ($F(2, 620) = 1.7$, $p = 0.153$). Wing shape between treatments ($F(68, 2432) = 3.72$, $p < 0.001$) differed significantly in late-starved drones from control or early starved ones (Fig. 1b, c). All the colonies had a highly significant difference in wing shape ($F(68, 2432) = 166.64$, $p < 0.001$). A significant interaction between the effect of starvation and colony was also found ($F(136, 4843) = 2.38$, $p < 0.001$). Procrustes FA score neither differed between experimental groups ($F(2, 620) = 0.70$, $p = 0.496$) (Tab. 1a) nor between colonies ($F(2, 620) = 0.57$, $p = 0.564$) (Tab. 1b) and no interaction between these factors was found ($F(4, 620) = 1.83$, $p = 0.122$).

DISCUSSION

Acute starvation during larval development had a significant negative effect on adult body mass and wing size. Starvation also changed the shape of drone forewings but not the volume of produced semen or asymmetry measures of forewings. It particularly influenced six-day-old larvae. The effect of early and late starvation differed probably because nursing bees feed the larvae differently according to age, needs (Haydak, 1970) and the colony's status (Mazeed, 2011).

Chemical analysis showed that there is a substantial difference in food provisioned to 2- and 6-day-old drones (Matsuka et al., 1973). Freshly hatched larvae receive from the nursing bees, large amounts of royal jelly but less caloric and less frequently. Drone larvae are later fed more caloric food (Matsuka et al., 1973) and visited more often by the nursing bees (Haydak, 1970).

Starved larvae are can also be fed more often after a period of food shortage in order to compensate the earlier lack of food (Huang & Otis, 1991). But Huang and Otis (1991) suggest that feeding increased with only up to four hours of larval starvation, while longer periods did not trigger further inspection or feeding from nursing bees. In our experiment, the larvae were starved for ten hours, but this period could be effectively shorter for mass provisioned younger larvae than for progressively provisioned older larvae.

Although body mass at emergence decreased through starvation in our study it still corresponded to average drone mass reported in literature ranging from 200 - 290 mg (Jay, 1963; Woyke, 1978; Gençer & Firatli, 2005; Mazeed, 2011, Szentgyörgyi et al., 2016). Semen volume, which is vital for drone fitness, was unaffected by starvation. We did not count the spermatozoa in the ejaculate, but earlier studies suggest that even if the amount of ejaculate is reduced, its quality remains stable even if body size deviates (Schlüns et al., 2003; Czekońska et al., 2015). Nevertheless, higher body mass may be an advantage during mating flights (Hrassnig et al., 2005) regardless of produced semen quality or quantity.

As expected, body mass at emergence and wing size were found to be strongly correlated (Es'kov & Es'kova, 2013). Wing size also differed between starved and control groups in a similar fashion as body mass, and late-starved (smallest) drones decrease in size the most. The lack of differences in the asymmetry of wing size and shape depending on the nutritional status of the larvae show that acute starvation did not change the conformation of wing venation independently for the left and the right side. High developmental stability of drone wing venation can result from strong selection for flight performance in drones. This is consistent with a recent study on chronic malnutrition of honey bee drones and workers during larval development, which also failed to produce clear differences in honey bee forewing fluctuating asymmetry (Szentgyörgyi et al., 2016). Wing shape asymmetry also showed similar levels in all groups, regardless of treatment or colony, and comparable to the ones reported by Łopuch & Tofilski (2016) and also by Szentgyörgyi et al., (2016). The observed lack of influence on wing development suggests that the short-term stress of starvation during early and late larval stage, although decreased body mass, was compensated and generally did not affect wing development taking place during the pupal stage.

In our analysis of wing asymmetry between the left and right wings for each individual we found a clear directional asymmetry (Palmer, 1994) of wing size, similarly to other studies (Schneider et al., 2003) in favor of the right side (Smith et al., 1997; Szentgyörgyi et al., 2016) and a significant difference in shape as described by Schneider et al. (2003), Smith et al. (1997) and Szentgyörgyi et al. (2016). Large changes 3 - 37% in wing area asymmetry, like clipping the distal-trailing edge of one wing are known to lower hovering-flight capacity, wing-tip velocity and aerodynamic reserve capacity (Vance & Roberts, 2014). In our case the directional asymmetry between the two sides was found to be less than 0.5 % of the calculated wing centroid size and probably did not cause significant differences in flying abilities. This may be the result of a yet unknown factor acting regularly during development and probably has no evolutionary meaning, other than simply a characteristic of the species.

We conclude that acute 10-hour starvation results in decreased body size of emerging drones but does not affect the volume of ejaculated semen. In drone honey bees, fluctuating asymmetry of the forewing is not a reliable measure of acute nutritional stress during larval development. Other such features as body mass or wing size are better indicators of starvation, since they can be used by honey bee queen breeders to control drone quality.

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REFERENCES

Alaux, C., Ducloz, F., Crauser, D., & le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, *6*, 562-565. http://doi.org/10.1098/rsbl.2009.0986

Babendreier, D., Kalberer, N., Romeis, J., Fluri, P., Bigler, F. (2004) Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie 35,* 293–300, http://doi.org/10.1051/apido:2004016

Berg, S. (1991). Investigation on rates of large and small drones at a drone congregation area. *Apidologie, 22*, 437-438.

Berg, S., Koeniger, N., Koeniger, G., & Fuchs, S. (1997). Body size and reproductive success of drones (*Apis mellifera* L). *Apidologie*, *28***,** 449–460. https://doi.org/10.1051/apido:19970611

Boes, K. E. (2010). Honeybee colony drone production and maintenance in accordance with environmental factors: an interplay of queen and worker decisions. *Insectes Sociaux, 57,* 1-9. https://doi.org/10.1007/s00040-009-0046-9

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie, 41*, 278–294. https://doi.org/10.1051/apido/2010012

Brückner, D. (1976). The influence of genetic variability on wing symmetry in honeybees (*Apis mellifera*). *Evolution, 30,* 100-108. https://doi.org/10.1111/j.1558-5646.1976.tb00886.x

Clarke, G. M., Brand, G. W., & Whitten, M. J. (1986). Fluctuating asymmetry: A technique for measuring developmental stress caused by inbreeding. *Australian Journal of Biological Sciences, 39*, 145-153. https://doi.org/10.1071/BI9860145

Cobey, S. W., Tarpy, D. R., & Woyke, J. (2013). Standard methods for instrumental insemination of *Apis mellifera* queens. *Journal of Apicultural Research, 52*, 1-18. http://dx.doi.org/10.3896/IBRA.1.52.4.09

Corby-Harris, V., Jones, B. M., Walton, A., Schwan, R. M., Anderson, K. E. (2014). Transcriptional markers of sub-optimal nutrition in developing *Apis mellifera* nurse workers. *BMC Genomics,15*, 134. https://doi.org/10.1186/1471-2164-15-134.

Couvillon, M. J., Hughes, W. O. H., Perez-Sat, J. A., Martin, S. J., Roy, G. G. F., Ratnieks, F. L. W. (2010). Sexual selection in honey bee: colony variation and the importance of size in male mating success. *Behavioral Ecology, 21*, 520-525. https://doi.org/10.1093/beheco/arq016

Crailsheim, K., & Hrassnigg, N. (1998). Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies. *Journal of Insect Physiology, 44*, 929–939. https://doi.org/10.1016/S0022-1910(98)00058-4

Czekońska, K., Chuda-Mickiewicz, B., & Samborski, J. (2015). Quality of honeybee drones reared in colonies with limited and unlimited access to pollen. *Apidologie, 46,* 1-9. https://doi.org/10.1007/s13592-014-0296-z

Czekońska K., & Chuda-Mickiewicz B., (2015). The ability of honey bee drones to ejaculate. *Journal of Apicultural Science, 59*(2), 127-133. https://doi.org/10.1515/jas-2015-0027

Dryden, I. L., & Mardia, K. V. (1998). *Statistical Shape Analysis*. Chichester: John Wiley and Sons.

Es'kov, E. K., & Es'kova, M. D. (2013). Factors influencing wing size and body weight variation in the Western honey bee. *Russian Journal of Ecology, 44*, 433-438. https://doi.org/10.1134/S1067413613050056

Graham, J. H., Raz, S., Hel-Or, H., & Nevo, E. (2010). Fluctuating asymmetry: methods, theory, and applications. *Symmetry, 2*, 466–540. https://doi.org/10.3390/sym2020466

Gençer, H. V., & Firatli, Ç. (2005). Reproductive and morphological comparisons of drones reared in queenright and laying worker colonies. *Journal of Apicultural Research, 44,* 163–167. https://doi.org/10.1080/00218839.2005.11101172

Grønkjær, P., & Sand, M. K. (2003). Fluctuating asymmetry and nutritional condition of Baltic cod (*Gadus morhua*) larvae. *Marine Biology, 143*, 191-197. https://doi.org/10.1007/s00227-003-1064-1

Haydak, M. H. (1970). Honey bee nutrition. *Annual Review of Entomology, 15*, 143-156. https://doi.org/10.1146/annurev.en.15.010170.001043

Hoover, S. E. R., Higo, H. E., & Winston, M. L. (2006). Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *Journal of Comparative Physiology B, 176*, 55-63. https://doi.org/10.1007/s00360-005-0032-0

Hrassnigg, N., & Crailsheim, K. (2005). Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie, 36*, 255–277. https://doi.org/10.1051/apido:2005015

Hrassnigg, N., Brodschneider, R., Fleischmann, P.H., & Crailsheim K. (2005). Unlike nectar foragers, honeybee drones (*Apis mellifera*) are not able to utilize starch as fuel for flight. *Apidologie 36*, 547-557. https://doi.org/10.1051/apido:2005042

Huang, Z-Y., & Otis, G. W. (1991). Inspection and feeding of larvae by worker honey bees (Hymenoptera: Apidae): Effect of starvation and food quantity. *Journal of Insect Behavior, 4,* 305-317. https://doi.org/10.1007/BF01048280

Human, H., Brodschneider R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, ... Zheng, H-Q. (2013). Miscellaneous standard methods for *Apis mellifera* research. *The COLOSS BEEBOOK* Volume I: standard methods for *Apis mellifera* research. (eds. V. Dietemann, J.D. Ellis, P. Neumann), *Journal of Apicultural Research, 52*(4), http://dx.doi.org/10.3896/IBRA.1.52.4.10.

Imdorf, A., Rickli, M., Kilchenmann, V., Bogdanov, S., Wille, H. (1998). Nitrogen and mineral constituents of honey bee worker brood during pollen shortage. *Apidologie, 29*, 315–25. https://doi.org/10.1051/apido:19980402

Jaffé, R., & Moritz, R. F. A. (2010). Mating flights select for symmetry in honeybee drones (*Apis mellifera*). *Naturwissenschaften, 9*, 337-343. https://doi.org/10.1007/s00114-009-0638-2

Jay, C. S. (1963). The development of honeybees in their cells. *Journal of Apicultural Research, 2,* 117–134. http://dx.doi.org/10.1080/00218839.1963.11100072

Jones, J., Helliwell, P., Beekman, M., Maleszka, R., Oldroyd, B. P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *Journal of Comparative Physiology A, 191,* 1121–1129. https://doi.org/10.1007/s00359-005-0035-z

Klingenberg, C.P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources, 11*, 353–57. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Köppler, K., Vorwohl, G., & Koeniger, N. (2007). Comparison of pollen spectra collected by four different subspecies of the honey bee *Apis mellifera*. *Apidologie, 38*, 341–53. https://doi.org/10.1051/apido:2007020

Kunert, K., & Crailsheim, K. (1985). Seasonal changes in carbohydrate, lipid and protein content in emerging worker honeybees and their mortality. *Journal of Apicultural Research, 27,* 13–21. http://dx.doi.org/10.1080/00218839.1988.11100775

Łopuch, S., & Tofilski, A. (2016). The relationship between asymmetry, size and unusual venation in honey bees (*Apis mellifera*). *Bulletin of Entomological Research, 106*, 304-13. https://doi.org/10.1017/S0007485315000784

Mattila, H. R., & Otis, G. W. (2006). The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee workers. *Apidologie, 37*, 533-546. https://doi.org/10.1051/apido:2006037

Matsuka, M., Watabe, N., & Takeuchi, K. (1973). Analysis of the food of larval drone honeybees. *Journal of Apicultural Research, 12,* 3-7. http://dx.doi.org/10.1080/00218839.1973.11099724

Mazeed, A. M. (2011). Morphometry and number of spermatozoa in drone honeybees (Hymenoptera: Apidae) reared under different conditions. *European Journal of Entomology, 108*, 673-676. https://doi.org/10.14411/eje.2011.085

Odoux, J-F., Feuillet, D., Aupinel, P., Loublier, Y., Tasei, J-N., Mateescu, C. (2012). Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies. *Apidologie, 43*, 561–575. https://doi.org/10.1007/s13592-012-0125-1

Ohlsson, T., & Smith, H. G. (2001). Early Nutrition Causes Persistent Effects on Pheasant Morphology. *Physiological and Biochemical Zoology, 74*, 212–218. https://doi.org/10.1086/319657

Ondo Zue Abaga, N., Alibert, P., Dousset, S., Savadogo, P. W., Savadogo, M., Sedogo, M. (2011). Insecticide residues in cotton soils of Burkina Faso and effects of insecticides on fluctuating asymmetry in honey bees (*Apis mellifera* Linnaeus). *Chemosphere, 83*, 585–592. https://doi.org/10.1016/j.chemosphere.2010.12.021

Palmer, A. R. (1994). Fluctuating asymmetry analyses. In: *Developmental Instability: Its Origins and Evolutionary Implications, Contemporary Issues in Genetics and Evolution 2* (pp 335–364). Netherlands: Springer.

Pernal S.F., & Currie R.W. (2000) Pollen quality of fresh and 1-year-old singel pollen diets for worker honey bees (*Apis mellifera L.*). *Apidologie, 31,* 387-409. https://doi.org/10.1051/apido:2000130

Schlüns, H., Schlüns, E. A., van Praagh, J., & Moritz, R.F. (2003). Sperm numbers in drone honeybees (Apis mellifera) depend on body size. *Apidologie, 34,* 577-584. https://doi.org/10.1051/apido:2003051

Schneider, S. S., Leamy, J., Lewis, A., & Degrandi-Hoffman, G. (2003). The influence of hybridization between African and European honeybees, *Apis mellifera*, on asymmetries in wing size and shape. *Evolution, 57*, 2350–2364. https://doi.org/10.1111/j.0014-3820.2003.tb00247.x

Schmickl, T., & Crailsheim, K. (2001). Cannibalism and early capping: strategy of honeybee colonies in times of experimental pollen shortages. *Journal of Comparative Physiology A, 187*, 541-547. https://doi.org/10.1007/s003590100226

Schmickl, T., & Crailsheim, K. (2002). How honeybees (*Apis mellifera* L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. *Behavioral and Ecological Sociobiology, 51,* 415–25. https://doi.org/10.1007/s00265-002-0457-3

Scofield, H. N., & Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS ONE*, *10*(4), e0121731. https://doi.org/10.1371/journal.pone.0121731

Smith, D. R., Crespi, B. J., & Bookstein, F. L. (1997). Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. *Journal of Evolutionary Biology, 10*, 551–574. https://doi.org/10.1046/j.1420-9101.1997.10040551.x

StatSoft Inc (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.

Szentgyörgyi, H., Czekońska, K., & Tofilski, A. (2016). Influence of pollen deprivation on the forewing asymmetry of honeybee workers and drones. *Apidologie, 47*(5), 653-662. https://doi.org/10.1007/s13592-015-0415-5

Taha, E. L. K. A., & Alqarni, A.S. (2013). Morphometric and reproductive organs characters of *Apis mellifera jemenitica* drones in comparison to *Apis mellifera carnica*. *International Journal of Scientific and Engineering Research, 4,* 411-415.

Tofilski, A. (2004). DrawWing, a program for numerical description of insect wings. *Insect Science, 4*, 17. https://doi.org/10.1673/031.004.1701

Wang, Y., Kaftanoglu, O., Fondrk, M. K., & Page, R.E., Jr (2014). Nurse bee behaviour manipulates worker honeybee (*Apis mellifera* L.). *Animal Behavior, 92*, 253–261. https://doi.org/10.1016/j.anbehav.2014.02.012

Woyke, J. (1978). Comparative biometrical investigation on diploid drones of the honeybee III. The abdomen, and weight. *Journal of Apicultural Research, 17,* 206-217. http://dx.doi.org/10.1080/00218839.1978.11099928

Zaitoun, S., Al-Majeed Al-Ghzawi, A., & Kridli, R. (2009). Monthly changes in various drone characteristics of *Apis mellifera ligustica* and *Apis mellifera syriaca*. *Entomological Science*, *12,* 208–214. https://doi.org/10.1111/j.1479-8298.2009.00324.x