

Characterization of Natural Bee Space and Cell Dimensions of Honeybees of Central Ethiopia (*Apis mellifera bandasii*)

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Abstract: *Apis mellifera* (*A. mellifera*) colonies build nests of varying characteristics which differ between races and different ecologies. To gain insight into details of natural nest characteristics of local honeybees and its implications in movable frame hive designing, natural bee space, comb spacing and worker brood cell dimensions of *Apis mellifera bandasii* (*A. m. bandasii*) were assessed across different altitudinal ranges through measuring their dimensions from naturally constructed combs in traditional hives. Moreover, comb spacing and bee space in movable frame hives from different local workshops were also measured. In addition, optimum comb thickness and bee space requirement were assessed by keeping local honeybee colonies in Kenya Top Bar (KTB) hives with different top bar widths. Accordingly, the average natural bee space, comb spacing, inside width of worker brood cells and worker brood cell/dm² were 10.04 ± 2.23 mm, 33.70 ± 1.84 mm, 4.64 ± 0.13 mm and 1060.92 ± 14.24 , respectively. The worker brood comb thickness, cell depth and bee space were significantly ($p < 0.05$) different across altitudinal ranges. Moreover, local honeybee colonies under natural conditions were found to build significantly narrower comb spacing in their brood nests than the frame spacing in movable frame box hives. The result from experimental colonies kept in KTB hives of varying top bar widths revealed that increasing comb spacing had no effect on comb thicknesses, while it significantly increased the bee space. This also confirmed that *A. m. bandasii* needs less comb spacing than the space in movable frame box hives currently used. The study implies the vital importance of considering natural nest comb spacing and cells dimensions in designing and constructing movable frame box hives and accessories that match with local honeybees' biological requirement which may contribute to enhance the productivity of the race.

Keywords: *Apis mellifera bandasii*, Bee Space, Comb Spacing, Cell Dimensions, Traditional Hive

1. Introduction

One of the most striking features of honeybee biology is the variability found within and between races of *A. mellifera*. Races of honeybees vary in morphological and natural nest characteristics. This natural variability is not surprising, considering that many bee races have been evolved in response to the diverse ecological conditions of their respective natural habitats [1]. The environmental differences between areas have led to speculations that honeybees in different areas, or of different genotypes, could differ in their nest requirement [2]. In traditional hives, honeybees build their natural nest by constructing a group of parallel combs vertically downwards from the roof of nest cavity almost the same way as they did in wild nests [3], which is not the case in movable frame hives. The

space they leave between the combs and comb spacing (midrib to midrib distances), and the variation in comb dimensions are inbuilt behavioral characteristics of the bees [3], which vary for subspecies based on their body sizes [4]. These variations are among the important factors affecting the performance and survival of *A. mellifera* honeybee races [2, 5], as the worker bees detect the spacing very precisely especially in brood combs, where nest temperature is crucial [6].

Feral honeybee colonies characteristically began comb building from one point and develop other combs on each side keeping their natural bee space and inter-comb distance [7]. The bee space is a path or corridor between comb surfaces, and between combs and walls of wild nest allowing at least two bees to walk and work freely on the comb surfaces [7, 8]. The bee space varies from 6 to 10 mm for *A. mellifera* races

depending on their body sizes [9]. In movable frame hives, bee space is needed between top bars of adjacent frames, between the outside end of each frame and inner hive wall opposite to it, and between the top of frames in the lower box and the bottom of the frames in the upper box. Like the bee space, comb spacing, the center to center distances between adjacent combs built in the wild nests, differs for various honeybee races depending on the body size of worker bees [10]. For instance, for European bee races, it ranges from 32 to 38 mm with an average of 35 mm, while for African bees it varies from 30 to 34 with the average of 32 mm [9]. These variations indicate that comb spacing might be determined by the size of the worker bees of a particular race, which, in turn, dictates frame bar width (frame spacing) in movable frame box hives, and the bar width in top-bar hives. Thus, bee space, comb spacing and worker cell dimensions in natural nests are vital factors to be considered while developing or adopting movable frame box hives to fit the natural requirement of a given bee race [11].

On the contrary, the types of movable frame box hives and their accessories used to keep local honeybees in Ethiopia are the ones that designed for European bee races without considering the biology and ecology of local bee races. However, lack of consideration for the biology and ecology of local bee races when adopting movable frame box hives and their accessories might affect the performances of the bees and rate of acceptance of the technologies in the country [11]. Hence, generating a detailed and tangible information on natural nest characteristics like the optimum bee space, comb spacing, and worker brood cell dimensions of *A. m. bandasii* is encouraged to enhance the understanding of its implications in movable frame hive and casting mold designing for local honeybees, which remains lacking. Therefore, the aim of this work was to assess the natural bee space, comb spacing, and worker brood cell dimensions of *A. m. bandasii* and compare with other races, as well as the spacing in movable frame box hives commonly used in Ethiopia.

2. Materials and Methods

This study was conducted in West Shoa and East Wellega Zones of Oromia Regional State where *A. m. bandasii* is supposed to exist [12]. For representative sampling, stratified purposive sampling techniques based on altitudinal ranges was employed, and each study zone was grouped into three altitude ranges (< 2000, 2000 – 3000, and > 3000 m above sea level).

Natural bee space: As searching and finding wild honeybee colonies and their nests is not easy in the two zones, the natural bee space of local honeybees was measured from traditional hives that are commonly used in the areas. The average natural bee space of local honeybee colonies was measured as the distance between two adjacent opposite brood combs built in the traditional hives. For this purpose, a total of 18 traditional hives with fully drawn brood combs were used per zone. For each hive, five bee spaces, a total of 180 measurements at different points were measured using digital caliper with the precision of 0.01 mm. To compare the bee space between naturally built combs and in movable frame box hives, bee space left in box hives from seven local workshops were also measured. Accordingly, five box hives were randomly taken from each workshop and five bee spaces per hive with a total of 175 bee spaces were measured (Figure 1).

Comb spacing: Comb spacing of a naturally constructed combs in traditional hives for local honeybees were measured between midribs of adjacent combs. Accordingly, measurements were taken from 10 different colonies of different localities within altitude range of 2000 – 3000 m. Five comb spacing (midrib to midrib distances) were measured for each colony (Figure 2). In addition, the average frame spacing in box hives constructed at seven different local workshops was estimated based on side bar width of the frames (spacer) in 70 box hives (Figure 1). For each box hive, 10 frames were measured for their side bar width. The results were compared with the results from naturally built comb spacing in traditional hives.



Figure 1. Measuring bar width of a frame (frame spacing, left) and bee space between two top bars of adjacent frames in movable frame box hive (right) (Photographed by author).

Comb thickness: The thicknesses of naturally built combs that has been used for rearing brood of worker bees by local honeybee colonies in the traditional hives were measured to estimate the thickness of the combs in the natural nests. Worker brood combs were obtained from the brood nests of

36 different colonies. The thicknesses of the combs were determined by measuring the thicknesses of five worker brood combs per hive.

Worker brood cell depth: Brood combs were obtained from traditional hives to determine the average cell depth of

worker brood cells. To measure the depth easily, worker brood combs with emerging bees from the center were taken and at least the depth of 50 worker cells were measured for each honey colony.

Worker cells density per unit area: The average density of worker cells per unit area was established as the number of

worker brood cells/dm² on naturally built combs (on both sides). To assess the cell density, naturally built worker combs were taken from 10 colonies in traditional hives at different localities only within the altitude range of 2000 – 3000 m. The cell count was made by placing a clear sheet of plastic to mark the area and this was done in three replications per colony.



Figure 2. Dissected traditional hive with fully built combs made ready for measuring natural bee space and comb spacing (left), measuring comb spacing in traditional hive (right).

Worker cell width: The inside width of each worker brood cell was measured with a caliper. A measurement was made for each cell, corresponding to the distance between the parallel sides of the hexagon cell. In such a way, 25 cells were examined in each colony and a total of 250 cells were measured for 10 different colonies.

Besides assessing natural bee space, comb spacing, comb thickness and cell dimensions, on station experiment was also conducted at Holeta Bee Research Center to see the effect of increasing comb spacing (top bar width) on the thickness of the worker brood combs and bee space left between combs of local honeybees. For this purpose, 18 KTB hives with the predetermined top bar width of 31, 32, 33, 34, 35 and 36 mm were constructed based on the results of comb spacing in natural nests. Honeybee colonies of similar strength were transferred to each hive at the start of active season to build combs in the same manner as they did in traditional hives. All the necessary management practices were done to the colonies. The thicknesses of 15 worker brood combs per hive was measured as for the traditional nests and their bee spaces were calculated from the measured comb thicknesses and the predetermined comb spacing (top bar width) as follows:

BS = CS - CT, where CS = predetermined comb spacing

CT = measured comb thickness

BS = Calculated bee space. Observation was also made for any burr comb and undrawn-out side of a comb due to larger or smaller comb spacing, respectively.

Statistical analysis: Comparisons of means were done with *t*-test and General Linear Model (GLM) procedures using IBM SPSS Statistics version 20. Tukey honest significance difference (HSD) at 95% confidence interval ($\alpha = 0.05$) level of significance was employed for mean separation.

3. Results

Natural bee space: The average natural bee space between combs built by local honeybees in traditional hives was 10.04

± 2.23 mm with a range of 5.00 - 13.82 mm ($N = 180$). The average natural bee space significantly varied among local honeybees of various altitude ranges ($p < 0.001$) (Table 1). Honeybees from higher altitude (> 3000 m) built combs with a wider bee space compared to bees from altitudes less than 3000 m. However, there was no significant differences between the natural bee space and bee space obtained from movable frame box hives constructed at different workshops ($t(353) = 1.08, p > 0.28$).

Comb spacing: The average comb spacing (midrib to midrib distances) of naturally built local honeybee workers' brood combs was 33.70 ± 1.84 mm with a range of 28.86 - 36.76 mm ($N = 50$) and the result was not significantly different among honeybee colonies ($F = 2.047, df = (9, 40), p > 0.059$). However, comparing the comb spacing of naturally built worker brood combs with a measured frame spacing in movable frame box hives, local honeybees had built significantly narrower comb spacing in their natural brood nest (33.70 ± 1.84 mm) than the frame spacing in box hives constructed at different workshops (39.01 ± 1.59 mm), $t(748) = 22.61, p < 0.001$. The measured frame spacing in box hives was not only significantly different from naturally built comb spacing, but also there was high significant differences among box hives from different local workshops ($F = 103.58, df = (6, 693), p < 0.001$).

Comb thickness: The overall average thickness of local honeybee workers' brood combs in natural nests from the three altitude ranges was 23.71 ± 0.30 mm with a range of 19.26 - 36.40 ($N = 180$). There was a significant variation in brood comb thickness among honeybee colonies from three altitude ranges ($p < 0.001$) (Table 1). Honeybees at higher altitude (above 3000 m) built thicker combs than the honeybees at altitude less than 3000 m.

Local honeybee colonies kept in the KTB hives of different top bar widths (31, 32, 33, 34, 35 and 36 mm), and their comb thicknesses and bee spaces are presented in Table 2. The thickness of the combs was not significantly different ($p > 0.369$) among the colonies kept in KTB hives

of different top bar widths. Although the bees built combs of similar thickness in all hives of different top bar widths, there was no undrawn-out side of a comb or a burr comb built because of the varying bar widths. Unlike comb thickness, the bee space left between the adjacent combs were significantly ($p < 0.001$) varied among colonies kept in hives of different top bar widths. The average bee space increased as the width of the top bars increased without significant increment in comb thickness. Generally, colonies kept in KTB hives with 36 mm bar width relatively

left larger space between adjacent combs (13.50 ± 0.88 mm) (Figure 2).

Worker cells density per unit area: The average number of worker brood cells/dm² of both sides of the naturally built combs was 1060.92 ± 14.24 with a range of $1032.52 - 1084.15$ cells ($N = 30$). Variations in the number of worker brood cells/dm² were not significant between locations and among local honeybee colonies kept in traditional hives at p value of 0.75 and 0.37, respectively.

Table 1. Mean \pm standard deviation of natural bee space, worker brood comb thickness and cell depth of honeybee colonies across altitudes.

Altitude range	Bee space (mm)	Worker brood comb thickness (mm)	Worker brood cell depth (mm)
< 2000 m	8.93 ± 2.52^b	21.14 ± 3.01^b	10.39 ± 0.18^b
2000-3000 m	9.60 ± 2.08^b	22.84 ± 2.39^b	12.57 ± 0.18^a
> 3000 m	12.49 ± 2.10^a	26.45 ± 3.03^a	12.56 ± 0.16^a
Grand mean	10.04 ± 2.23	23.48 ± 3.58	11.13 ± 0.16

Means with different superscript letters in a column are significantly different at $p < 0.05$.

Worker brood cell dimensions: The average inside width and depth of natural worker brood cells were 4.64 ± 0.13 ($N = 250$) and 11.31 ± 0.16 mm ($N = 1126$), respectively. The cell width of worker brood cells significantly varied among

local honeybee colonies of different locations ($p < 0.001$). Likewise, the result for the depth of naturally built worker brood cells had shown high significant differences when compared among altitude ranges ($p < 0.001$) (Table 1).

Table 2. Mean \pm standard deviation of worker brood comb thickness and bee space left in colonies kept in KTB hives of different top bar widths.

Top bar width (mm)	Worker brood comb thickness in mm (Mean \pm SD)	Calculated bee space in mm (Mean \pm SD)
31	21.81 ± 0.99^a	9.14 ± 0.99^d
32	22.04 ± 1.22^a	9.96 ± 1.22^d
33	21.60 ± 1.08^a	11.40 ± 1.08^c
34	21.86 ± 0.84^a	12.14 ± 0.84^{bc}
35	22.00 ± 1.49^a	12.99 ± 1.49^{ab}
36	22.50 ± 0.88^a	13.50 ± 0.88^a
P value	0.369	0.001

Mean values with different superscript letters within a column are significantly different at $p < 0.05$.

4. Discussion

Apis mellifera colonies construct nests of varying characteristics, which differ from ecology to ecology and race to race [2, 13]. The presence of variations in bee space among different races and altitude ranges is among the varying nest characteristics [9, 14]. In this result, the natural bee space left between adjacent combs was significantly varied across altitude ranges. Honeybees at higher altitude (> 3000 m) were found to build combs with a larger bee space than the honeybees at lower altitude (< 3000 m), which is consistent with the previously reported bee space across altitude for *A. m. scutellata* [14]. The large bee space at higher altitude suggests that the bee space is associated with the body size of honeybees [9] as mountain bees of the same subspecies could have relatively larger body size [8, 15], which in turn, need more space to move freely compared to honeybees with smaller body size. Regarding the overall average natural bee space of *A. m. bandasii* of Ethiopian local honeybees in traditional hives (10.04 mm), it was found to be more than the natural bee space reported for *A. m. jemenitica* (7.27 mm) [11], whereas it is less than what has been reported for other *A. mellifera* race of northern Ethiopia [16]. Moreover, the non-significance differences between the

mean natural bee space and the space left between adjacent top bars of frames in movable box hives suggests that the bee space in frame hives might be in line with the natural requirement of local honeybees. This is in agreement with the finding that local honeybee races perform well in frame hives with bee space of 7 mm to 10 mm [17].

Comb spacing in a natural nest is the distance between the adjacent comb midribs [9]. This space varies from species to species, as well as within subspecies [10, 11, 18]. The average midrib to midrib comb distances of local honeybees was 33.70 ± 1.84 mm, which is much more than that of *A. m. capensis* (27.9 mm) and *A. m. jemenitica* (29.8 mm) [11, 19], and it is within the range of other African *A. mellifera* races (30 - 34 mm) [9]. However, it is smaller than the comb spacing used for European bee races (35 mm) [9]. This suggests the comb spacing might have some correlation with the size of honeybees [10]. Furthermore, the frame spacing of movable frame box hives (39.01 ± 1.59 mm) designed for temperate evolved races of *A. mellifera* and currently under use in the central part of the country was significantly larger than the result obtained for natural comb spacing. Actually, it is a known fact that temperate evolved European honeybees are considerably larger than our local bees [1, 10]. Variations in honeybees body size may be reflected in the dimensions of

cells built by the bees [1], which, in turn, determine the optimum comb spacing for a given race [9]. However, the width of frame space of movable frame box hives being used in the central part of the country is even larger than what has been recommended for temperate bees (35 mm). This difference and lack of consideration for the biology and ecology of the local bee race might affect the performances of local honeybee colonies, as well as the acceptance of the hive by local beekeepers [11]. Overall, these evidences support the previous recommendations of bee space and comb spacing suitable for tropical and sub-tropical African honeybee races [18, 21].

In natural nests, honeybees of a given race construct combs of almost uniform comb thickness in their brood nests [22] depending on the body size of adult workers [4]. The thickness of the combs and bee space left between the adjacent worker brood combs in wild nests of a race is a vital factor that determines their comb spacing [9]. The distance equals to the thickness of the comb plus the bee space required by the adult bees to crawl between opposite comb surfaces. In this study, the non-significant comb thicknesses and the increasing trend in bee space as the top bar width increased in the experimental colonies kept in KTB hives with varying top bar widths signify the vitality of body size in determining comb thickness. This agrees with the findings that honeybees of a given race build brood combs with almost uniform thicknesses which highly correlated with the body size of adult worker bees [4]. Moreover, similar results of uniform comb thickness across agro-ecology has been reported for *A. mellifera* from northern Ethiopian [16]. In contrast, the large variation in the bee space suggests that local *A. mellifera* workers are more tolerant of comb spacing factor [23]. However, comb spacing, which, in turn, affects the bee space in movable frame box hives is critical in maintaining optimal conditions within the brood nest. Worker bees need only just space for them to walk and work on the surface of the combs, while maintaining the optimum nest temperature [8].

Although *A. mellifera* workers are more tolerant to bee space factor, the provision of optimal bee space [21] is an important factor for thermoregulation which can be most efficiently achieved by just two layers of bees between the combs [8]. Under colder conditions more bees are recruited to occupy the between-comb space where they generate heat and slow the convective flow of air [24]. Wider comb spacing which resulted in larger bee space requires more layers of bees to ensure normal brood development. Under this condition, honeybee workers spend much energy to regulate and maintain brood nest temperature in the range of 32–36°C [24, 5], as lower temperatures than optimal have been reported to cause a number of negative developmental and behavior changes in honeybees [26]. For instance, short-term exposure of capped brood to very low temperature has been shown to increase pupal mortality, the incidence of miss orientation inside cells, and decrease workers longevity [27], which may finally affect performances of the colonies. On the contrary, the optimum space is suggested to positively

affect the heat regulation in the brood nest, increasing the rate of development of worker bees, as well as their number in the brood nest, which, in turn, might favor their performance by increasing the time spent on normal nest activities [28]. Thus, the uniform comb thickness in movable top bar hives with different bar widths and differences in the bee spaces with the increased bar width are indicative of an importance of comb spacing that might affect the suitability of movable frame box hives for local honeybees. This evidence also supports the previous finding [11] that the success of the modern hives depends on the fact that the bee space it provides is calculated according to the bee race used.

Under natural conditions (in the absence of foundation sheet), comb construction is notoriously variable: different races of *A. mellifera* construct worker cells of varying width at the rate of 500 to 1124.5 cells/dm² [9, 11, 29]. To this end, local honeybees (*A. m. bandasii*) in the study areas were observed to build cells with an average inner width (distance between two opposite cell walls) of 4.64 ± 0.13 mm with the range of 4.30 – 4.90 mm. This value is smaller than that of European *A. mellifera* races (5.2 mm) [22, 30, 31] and the naturally built worker cells of *A. m. adansonii* (4.80 mm) [29]. However, the current result is comparable to workers' cells width reported for other *A. mellifera* races of Africanized bees (4.48 mm) [30] and relatively wider than what has been reported for *A. m. jemenitica* (4.07 mm) [11], suggesting that worker cell size considerably depends on honeybee races. Moreover, the average depth of worker brood cells constructed by local honeybees (11.13 ± 0.16 mm) was deeper than that of *A. m. jemenitica* (9.39 mm) [11], while it was almost as deep as those of other *A. mellifera* races (11 mm) [22]. Furthermore, local honeybees were observed to build worker brood cells of 1060.92 ± 14.24 /dm², which is relatively more than cell density of European *A. mellifera* (830 cells/dm²) in commonly used marketed wax foundation [28]. However, it is much closer to cell density reported for other *A. mellifera* races [6, 29]. The result is also in agreement with the finding that African evolved *A. mellifera* bees naturally build more worker brood cells/dm² than the number of cells built on embossed foundation sheet designed for European bees [11]. This suggests that cell size on the foundation is determined by the size of the bees which may vary among bee races [6, 18]. Hence, these findings reveal that local honeybees (*A. m. bandasii*) has smaller natural worker cell dimensions than European *A. mellifera* and may need to develop casting molds with a suitable cell size that matches their natural cell size.

5. Conclusion

The study underlined the presence of variations in natural bee space, comb thickness, and cell size within *A. m. bandasii* colonies of different altitude ranges. Moreover, *A. m. bandasii* requires relatively smaller comb spacing and worker brood cell dimensions than European bee races. Furthermore, large variation between frame spacing in box hives designed for European bee races and natural comb spacing was

observed, which may affect the performances of local honeybees. In addition, the uniform comb thickness and varying bee space in experimental colonies kept in movable top bar hives with different bar widths suggest that increasing comb spacing above the natural limit may be a wastage of space and adds unnecessary work load to the bees. Hence, considering natural bee space, comb spacing, and cell dimensions are important to design and construct movable frame box hives that match with local honeybees' biological requirement rather than directly adopting of box hives and casting molds designed for European bees. However, a more detailed agro-ecologic based performance study of local honeybee races in movable frame box hives designed based on the new information from natural nests is required to design and develop movable frame hives and accessories that fit the requirement of the local honeybee race.

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