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The Influence of Size of Brood Cell Upon the Size and Variability of the Honeybee

(*Apis mellifera L.*)

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SUMMARY

1. Enlarged brood cells affect the size of the adult worker bee, and significantly larger worker bees are obtained through the use of enlarged cell foundation.

2. The average percentage increases of the linear measurements of the adult worker bees are almost proportional to the percentage increases of the diameters of the brood cells.

3. The increase in the size of the bee does not quite keep pace with increases in cell size.

4. Size of brood cell apparently does not affect the variability of the adult worker bee, except possibly its dry weight.

5. The number of bees used in a sample in this experiment is not great enough to give wholly consistent results, but these results in general are significant and indicative.

6. Dry weight is difficult to measure and further experimental control is needed.

7. Among body measurements, exclusive of proboscis, length of right forewing gives the best estimate of length of proboscis.

8. Of the two major parts of the proboscis, the mentum (which is the more easily measured) is more highly correlated with proboscis length than is the glossa.

9. A combination of length of right forewing and mentum length affords an excellent estimate of proboscis length.

The Influence of Size of Brood Cell Upon the Size and Variability of the Honeybee (*Apis mellifera L.*)[1]

By Roy A. Grout[2]

The problem of raising larger honeybees, especially those having longer "tongues" or a greater "tongue reach," has been a topic of interest in this country since the beginning of the present century. Increased "tongue reach" in the honeybee would be desirable because additional nectariferous flora would become available for honey production. It would be advantageous also to the agriculturist because of the increased seed production that would result in certain plants of economic importance, especially red clover, *Trifolium pratense*. Under present circumstances red clover produces very poor crops of seed throughout the Midwest because it is not able to compete successfully with short tubed flowers, such as sweet clover, *Melilotus sp.*, and White Dutch clover, *Trifolium repens*, for the pollinating services of the honeybee.

Baudoux (5), in Belgium, was the first to conceive of the use of an artificial foundation having an enlarged cell base to increase the size of the emerging bee. Pincot, in France, according to Gillet-Croix (13) and Lovchinovskaya (22), in Russia, also have carried out similar experiments. While the data presented by the first two cannot be considered of a very scientific nature, they have been convincing to the extent that certain manufacturing houses in Belgium and France and more recently in Italy and England are manufacturing artificial foundation having larger cell bases and claiming good results through its use. Consequently interest in this country has been focused upon this matter.

The purpose of this investigation is to study the influence of enlarged brood cells upon the size and variability of the emerging worker bee by measuring various characters of the individual bee. The writer realizes that the crucial test for the commercial use of enlarged foundation is honey production, but the present study should be a strong indication toward that end.[3]

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[1] Project 129, Iowa Agricultural Experiment Station.

[2] This paper is taken from a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, master of science.

The author wishes to express his sincere appreciation and gratitude to Dr. O. W. Park, professor of apiculture, Iowa State College, under whose direction this research was done; to George W. Snedecor, professor of mathematics, Iowa State College, for assistance in statistical interpretations; and to Henry C. Dadant for supplying the special foundation used in the experiment.

[3] The use of enlarged cell foundation in an apiary at Hamilton, Ill., during a 3-year period has shown nothing of significance either for or against its use as a factor in increasing honey production.

REVIEW OF LITERATURE

Huber (15), in 1791, at the suggestion of Bonnet, first investigated the effect of brood cells upon the size of the honeybee by rearing worker bees in drone cells and drone bees in worker cells. In the latter case he observed that the drone bees were smaller. Others who recorded having observed the phenomenon of worker brood reared in drone cells sealed with level cappings are Alley (1) in 1869; Berlepsch (7), in 1867; Gundelach, Gunther, Klempin, Zarudski, Zesselski, Lehzen and Hanneman, and Buttel-Reepen, according to Michailov (25); Pincot, according to Gillet-Croix (13); and Drory, according to Getaz (12). Of the above observers only Zarudski noticed any increase in the size of the worker bees due to their rearing in drone cells.

Microscopical investigations were first made by Martynov (23) in 1901, who showed that worker bees reared in drone cells had a proboscis length of 7.01 mm. as compared to 6.06 mm. in the case of their worker cell sisters. A similar and more extensive study was made by Michailov (25) in 1925, who measured six characters of the bee. He concluded that worker bees reared in drone cells were significantly larger and decidedly more variable than their worker cell sisters.

Further pursuit of the literature relating to the effect of size of brood cell upon the size and variability of the honeybee leads into several distinct controversies enumerated below.

1. LENGTH OF PROBOSCIS AND ITS RELATION TO HONEY STORING ABILITY

This controversy reached its peak in this country about the beginning of the present century when Root (32) determined the average "tongue reach" of a colony which was gathering nectar from red clover (*Trifolium pratense*) to be 0.21 inches, compared to an average tongue reach of 0.16 inches, and later sold the progeny of this queen as "red clover queens." Kulagin (20) procured four of these queens and records that the length of proboscis of their progeny was found to be 6.22 mm. as compared with an average of 6.21 mm. for the common black bees of Central Russia. Rankin (31) previously recorded being successful in breeding bees having longer tongues.

Previous to the controversy in this country, Wankler (38), in Germany, had already reported success in breeding bees having longer tongues and Charton (9), in France, had invented a glossometer and by 1897 had presented figures which indicated that bees stored in proportion to the length of their proboscides. More recently, in Germany, Zander (39), Tiedmann (35) and Götze (14) have further contributed, particularly the latter, who concluded that only those bees having the longest known proboscides can procure nectar from existing varieties of red clover.

In this country we must mention the work of Merrill (24), who in 1922 determined that a correlation between length of proboscis alone and storing ability could not be found but that the length of the proboscis plus carrying capacity and colony strength were highly correlated with yield. Hutson (16) in 1926, working with smaller numbers of bees, confirmed Merrill's results.

2. THE EFFECT OF THE AGE OF COMB UPON THE SIZE OF THE EMERGING BEE

The reduction of the size of the brood cell by the accumulation of the cast-off pupa skins, cocoons, excrement and varnishing resulting from each generation has been discussed by Quinby (30), Dadant (10) and others. All maintained that the lengthening of the side walls of the brood cell compensated for the slight thickening of the side walls and that the volume of the cell, if reduced, did not materially affect the size of the emerging bee.

Tuenin (36), upon weighing bees that had emerged in succeeding generations up to 48 generations and measuring the brood cells, showed a reduction in the weight of the bee and a corresponding reduction in the diameter of the cells. Michailov (26) continued this experiment by measuring five physical characters of the endo-skeleton and showed that after 16 to 18 generations a reduction of 5.89 percent occurred in the diameter of the cell with a significant reduction in the size of the bee. A reduction of 3 percent in the diameter of the cell showed no significant decrease in the size of the bee. Rupp (33) calculated, from the data obtained by Michailov, that a comb is too old for brood rearing when it is 3 years old.

3. ARTIFICIAL FOUNDATION HAVING AN ENLARGED CELL BASE

With the invention of artificial comb foundation by Mehring, in 1857, a control of the size of the cells constructed by worker bees was obtained. Various measurements of the cells resulted, by Collin, Langstroth and Charles Dadant, according to Dadant (11), Baudoux (6), Pincot, according to Gillet-Croix (13) Halleux, according to Szezawinski (34), and Baldensperger (4), recording a variation of from 764 to 940 cells per square decimeter for various races of bees.

Baudoux (5) was the first to advocate the use of artificial foundation having an enlarged cell base. Observing the reduction in the size of bees from an old skept containing combs having 912 cells per square decimeter, he conceived the idea of stretching artificial foundation in order to enlarge the cell base. Experimenting with artificial foundation having 750, 740, 730, 710, 700 and 675 cells per square decimeter, he concluded that bees reared in combs built from foundation having 700 cells per square decimeter were larger in all their measurements than those reared in combs built from smaller sizes.

Independent of the work done by Baudoux, Pincot, according to Gillet-Croix (13), experimented with foundation having 736 cells per square decimeter and recorded that during a 2-year period 30 colonies gathered approximately one-third more honey than did 30 colonies on normal foundation. Lovchinovskaya (22), reporting investigations started in 1925, concluded that bees from enlarged cells weighed more, that they had a greater load capacity and that, from the results of one season, they produced more honey.

4. STUDIES CONCERNED WITH VARIABILITY

Data have been presented concerning the variability of the honeybee by Michailov, Tuenin, Choclov and Alpatov, as cited by Alpatov (2), Koschevnikov (19), Landacre (21), Phillips (28 and 29), Casteel and Phillips (8), Bachmetjew (3), Pearl (27), Kellogg and Bell (18), and Kellogg (17). The researches of the latter showed that the variability of drones reared in worker cells was greater than drones reared in drone cells but that this greater variation was not due to special extrinsic factors such as size of cell.

EXPERIMENTAL

PURPOSE OF STUDY

As previously stated, the purpose of this experiment is to study the size and variability of the worker bee as influenced by its rearing in brood cells constructed by worker bees on artificial foundation having enlarged cell bases. Three sizes of foundation were used in this experiment, having 857, 763 and 706 cells per square decimeter respectively. The foundation having 857 cells per square decimeter is the standard commercial size manufactured in the United States, while the two latter sizes approximate that having 750 cells per square decimeter which has been manufactured since 1896 by Jos. Mees Sons of Herenthals, Belgium, and that having 700 cells per square decimeter which the same firm has manufactured since 1927.

METHODS AND MATERIALS

The foundation used in this experiment was furnished by Dadant and Sons, of Hamilton, Ill. The cutting of special dies and the manufacturing of the foundation was personally superintended by Mr. H. C. Dadant, particular attention being paid to the milling of the foundation in order that the resulting cell bases should be true hexagons. The foundation contained ten wires imbedded in the vertical position which, when placed in a frame wired with four horizontal wires, did not warp but resulted in perfect combs when drawn out by the bees.

To facilitate recognition and handling of the combs, the system used by Professor Park in marking the frames in a prior experiment was followed here. The frames containing the standard-size foundation, having 857 cells per square decimeter, were marked "A," and one notch was cut in the top bar. Frames containing the foundation having 763 cells per square decimeter were marked "B," and two notches were cut in the top bar, while the frames containing foundation having 706 cells per square decimeter were marked "C," and three notches were cut in the top bar.

No control of the size of cell other than special foundation was exercised. Frames containing all three sizes of foundation were placed in each of 23 colonies of the Iowa State College Apiary early in the summer of 1930. In general, two frames of each size were placed in each colony.

Individual colony records were kept and the queens were marked by clipping the right wings of those reared in an even-numbered year, and the left wings of those reared in an odd-numbered year.

An effort was made to collect the bees upon emergence from all three sizes of cells in a single colony at approximately the same time and under the same conditions. For this purpose a chart was made whereby the daily emergence of the bees from each size of cell was recorded for all of the 23 colonies. Each frame was caged in a Root Nucleus Introducing Cage a day or two before the time of emergence, and a selected area of brood was covered with an additonal small screen cage insuring that the emerging bees would have no access to any nectar or honey.

The sample collected from each brood comb contained at least 50 bees. During the summer of 1930, over 6,000 bees were collected. During June of 1931, over 600 bees were collected. From these, approximately 3,500 were selected as being most suitable for the experiment. The bees of this group were in sets of 150 bees, consisting of three samples of 50 bees each, taken from each of the three sizes of cells from the same colony, from the same mother and at approximately the same time.

After collection, the bees were slightly anesthetized, either with ether or calcium cyanide, and then killed by dropping into boiling water. This method of killing, as shown by Alpatov (2), caused the proboscis to be fully extended. The sample was then preserved in a 70 percent alcohol solution for further treatment.

The general plan of procedure for measuring the size of the individual bees of a sample was as follows: (1) Determining the weight of the individual bee. (2) Dissecting the right fore-wing, the third tergite, the fourth tergite and the proboscis of each individual bee. (3) Mounting these parts for measurement. (4) Measuring the parts.

Experiments showed that it was advisable to take the individual dry weight of each bee. The bees were removed from the 70 percent alcohol solution, dried on filter paper for several minutes to remove excess preservative and placed in a De Khotinsky Constant Temperature Oven Appliance at a constant temperature of 70 degrees C. for 48 hours. They were then placed in a desiccator containing concentrated sulfuric acid in its base for 72 hours, after which time no further appreciable loss of weight occurred.

The individual bees were then taken from the desiccator and weighed by means of an Eimer and Amend chemical balance accurate to 0.1 mg. A container of fresh calcium chloride was kept within the chemical balance at all times to dehydrate the contained atmosphere. A test sample was weighed at intervals during an extended series of weighings to determine the gain in weight of the individual bees due to the repeated opening of the desiccator. All weights given in this experiment are corrected for this factor.

After being weighed, each bee was placed in a numbered vial containing tap water at room temperature and throughout the following treatment was recognized as a definite individual. After remaining in the water for 24 hours, the bees were soft enough for dissection. With the aid of a Spencer Binocular Microscope containing a 3.5x ocular and a 55 mm. objective, and an ordinary dissecting set, the right forewing, the third tergite, the fourth tergite and the proboscis of each bee were dissected. The dissected parts were then mounted directly upon numbered glass slides with Bueston's medium,[4] and cover glasses were applied.

All linear measurements were taken by a projection method. The numbered glass slide was placed in a Leitz Simple Micro-Projector in a vertical position and projected upon a movable screen attached to the opposite wall. Upon the face of the

[4] Bueston's medium for mounting:

Water	50 c.c.
Glycerine	20 c.c.
Gum arabic	40 gm.
Chloral hydrate	50 gm.

Dissolve gum arabic in water. When dissolved, add chloral hydrate. When this is dissolved, add glycerine. Filter.

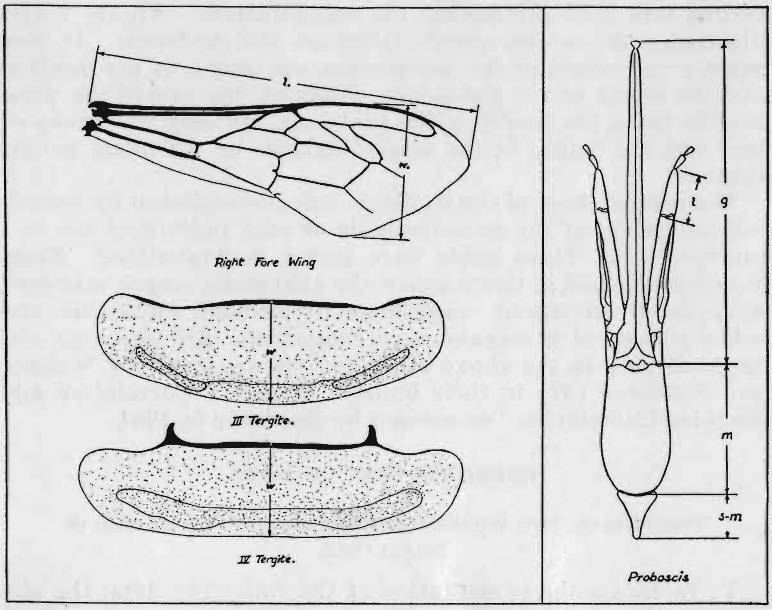

Fig. 1. Showing location of the linear measurements made on various parts of the honeybee. w.—width of part. l.—length of right forewing. g.—length of glossa. m.—length of mentum. s.m.—length of submentum. g. + m. + s.m.—length of proboscis.

screen were a horizontal and a vertical scale, and the screen was so constructed that the entire face could be rotated around its center in a plane perpendicular to the line of projection. This feature greatly facilitated measuring the projected parts since the measuring scale could be turned to any angle at which the part to be measured might happen to lie.

The projection measurement apparatus was adjusted so that the glass Spencer stage micrometer, having a scale 2 mm. in length ruled to 0.01 mm., placed in the micro-projector gave a corresponding projection of 2 mm. magnified 127 times on the scale of the movable screen. The apparatus was calibrated by this method before and at intervals during each long series of measurements. While it was possible to read directly the measurement of the part in hundredths of a millimeter, a reading was taken at the beginning of the part and another at its end, the true measurement being the difference between the two readings.

Figure 1 shows diagramatically the measurements taken on the right forewing, the third tergite and the fourth tergite. Following the system used by Michailov (25), the widths of the third and fourth tergites were combined and the sum of the two widths was used throughout the computations. Figure 1 also illustrates the measurements taken on the proboscis. In this manner the length of the submentum, the length of the mentum and the length of the glossa were obtained, the sum of the three lengths being the length of the proboscis. In only one group of bees was the length of the second member of the labial palpus taken.

The computation of the statistics was accomplished by recording the values of the measurements of each individual bee in a punched card. These cards were sorted and tabulated. From the sum obtained in this manner, the arithmetic means, standard deviations, correlation coefficients, regression equations and other statistical constants were computed. All formulae and methods used in the above computations are given by Wallace and Snedecor (37) in their bulletin entitled "Correlation and Machine Calculation" as revised by Snedecor in 1931.

PRESENTATION OF DATA

THE SIZE OF THE WORKER BEE AS INFLUENCED BY SIZE OF BROOD CELL

To facilitate the presentation of the following data, the size of the cell contained in a comb having 857 cells per square decimeter is designated as A; the size of cell contained in a comb having 763 cells per square decimeter is designated as B; in a similar manner the size of cell contained in a comb having 706 cells per square decimeter is designated C.

There is a reduction of 94 cells per square decimeter between A and B, while between B and C there is a reduction of 57 cells per square decimeter, making a total reduction of 151 cells per square decimeter between A and C. Corresponding increases in area are 12.30, 8.07 and 21.39 percent; in diameter, 5.98, 3.96 and 10.18 percent (table 2).

From the bees collected during the summer of 1930, data are presented here on bees from three colonies only. A sample of 50 bees was collected from colony 25 from an A comb on Aug. 21, 1930. On Aug. 28 two samples of bees were collected, one from a B and one from a C comb. The bees from colony 21 were collected within a period of 2 days, two samples being collected from an A and a B comb, respectively, on Aug. 18, 1930, and a third sample from a C comb on Aug. 20, 1930. The bees from colony 18 were collected over an extended period of time. One sample was taken from an A comb on Aug. 30, 1930, another from a C comb on Sept. 7, and the third from a B comb on Sept. 23. The individual hive records of these three colonies show that the bees from each colony were the progeny of one mother.

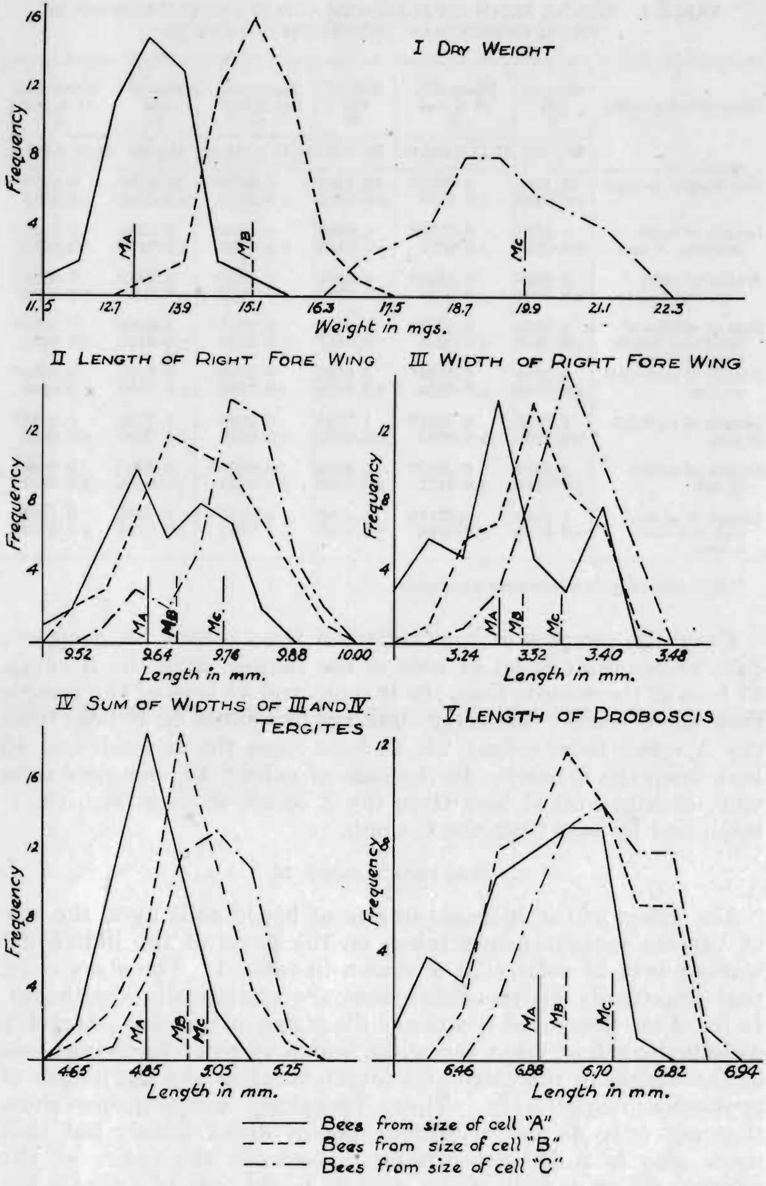

Fig. 2. Frequency distribution of the measurements of the characters dry weight, length of right forewing, width of right forewing, sum of the widths of the third and fourth tergites and the length of the proboscis.

TABLE 1. MEANS, MEAN DIFFERENCES AND STANDARD ERRORS OF MEASUREMENTS OF BEES FROM COLONY 25.

Measurement taken	Size of cell A	Mean diff. of A and B	Size of cell B	Mean diff. of B and C	Size of cell C	Mean diff. of A and C
	M ± δM.	M.D. ± δM.D.	M ± δM.	M.D. ± δM.D.	M ± δM.	M.D. ± δM.D.
Dry weight in mgs.	13.1000 ±0.0939	2.0302* ±0.1427	15.1302 ±0.1075	4.6876* ±0.2960	19.8178 ±0.2758	6.7178* ±0.2913
Length of right forewng. n mm.	9.6075 ±0.0229	0.0578* ±0.0277	9.6653 ±0.0155	0.0856* ±0.0234	9.7509 ±0.0175	0.1434* ±0.0288
Width of right forewing in mm.	3.2836 ±0.0109	0.0345* ±0.0141	3.3181 ±0.0090	0.0381* ±0.0120	3.3562 ±0.0079	0.0726* ±0.0135
Sum of widths of third and fourth tergites in mm.	4.8545 ±0.0148	0.1087* ±0.0223	4.9632 ±0.0167	0.0721* ±0.0235	5.0353 ±0.0165	0.1808* ±0.0222
Length of proboscis in mm.	6.5916 ±0.0190	0.0614* ±0.0224	6.6530 ±0.0118	0.0750* ±0.0188	6.7280 ±0.0146	0.1364* ±0.0240
Length of mentum in mm.	1.7477 ±0.0062	0.0261* ±0.0082	1.7738 ±0.0053	0.0020 ±0.0079	1.7758 ±0.0059	0.0281* ±0.0086
Length of glossa in mm.	4.2832 ±0.0147	0.0517* ±0.0172	4.3349 ±0.0090	0.0531* ±0.0152	4.3880 ±0.0123	0.1048* ±0.0192
Length of glossa and mentum in mm.	6.0316 ±0.0164	0.0771* ±0.0196	6.1087 ±0.0107	0.0551* ±0.0175	6.1638 ±0.0138	0.1322* ±0.0214

*Mean difference is statistically significant.

From the samples of bees collected from colony 25, complete data were obtained on 44 bees of the sample from the A comb, 47 bees of the sample from the B comb and 45 bees of the sample from the C comb. Similarly, data are presented on 40 bees from the A comb from colony 21, 43 bees from the B comb and 45 bees from the C comb. In the case of colony 18, complete data were obtained on 41 bees from the A comb, 48 bees from the B comb and 50 bees from the C comb.

BEES FROM COLONY 25

The effect of the increase in size of brood cells upon the size of various measurements taken on the parts of the individual worker bees of colony 25 is shown in table 1. The stars show that practically all the differences are statistically significant. In fig. 2 are presented frequency diagrams of the characters dry weight, length of right forewing, width of right forewing, sum of the widths of the third and fourth tergites and the length of proboscis, respectively. These frequency distributions show that not only do the arithmetic means differ widely but that there also is a distinct difference between the peaks of the curves. There is indicated a definite trend toward a larger bee as the size of cell is increased.

An interesting fact about this particular sample of bees is brought out by computing the ratio,

$$\frac{\text{average of seven linear measurements}}{\text{diameter of foundation cells}}$$

for the three sizes. The results are:

A, 1.02; B, 0.98; C, 0.95.

The small differences among these ratios show that the linear measurements of the bees increased almost proportionally with the diameter of the cells. The downward trend in the ratios, however, indicates that these measurements did not quite keep pace with the increasing diameter. The bee was unable to utilize completely the larger space available.

The percentage increase of the linear measurements on the various parts of the bees is shown diagramatically in fig. 3 and the percentage increases of all measurements are given in table 2.

BEES FROM COLONY 18

In table 3 are statistics on measurements of worker bees from colony 18. In the main they confirm those of colony 25, but with fewer significant differences. Curiously enough, the only significant difference between A and B is negative; the bees from the larger cells had the smaller dry weight.

It is to be expected, however, that the measurement of dry weight should be less consistent than the others. This is due to the difficulty of obtaining two or more samples in which the bees are in exactly the same condition with reference to such things as fecal matter and moisture content. It could have happened in this case, for instance, that the bees from the

TABLE 2. PERCENTAGE INCREASE OF MEASUREMENTS AS SIZE OF BROOD CELL INCREASES (COLONY 25).

Measurement taken	Percent increase from A to B	Percent increase from B to C	Percent increase from A to C
Dry weight	15.50	30.98	51.27
Length of right forewing	0.60	0.89	1.49
Width of right forewing	1.05	1.15	2.21
Sum of widths of third and fourth tergites	2.24	1.45	3.72
Length of proboscis	0.93	1.13	2.07
Length of mentum	1.49	0.11	1.61
Length of glossa	1.21	1.22	2.45
Sum of lengths of mentum and glossa	1.28	0.90	2.19
Average of seven linear measurements	1.12	1.06	2.19
Diameter of cell	5.98	3.96	10.18
Area of cell	12.30	8.07	21.39

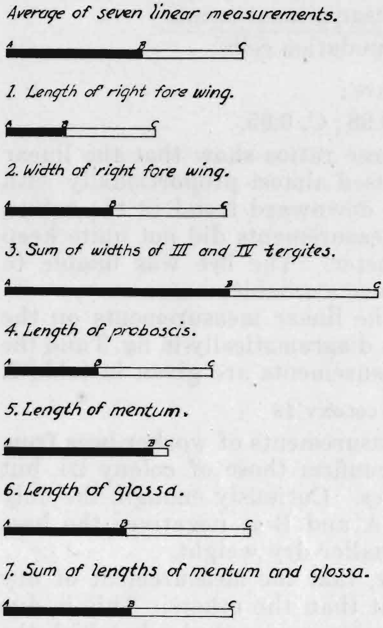

Fig. 3. Illustrating the percentage increases in the linear measurements obtained from honeybees reared in enlarged cells (sizes B and C) as compared with those from bees reared in normal cells (size A).

larger cells contained less fecal matter than those from the smaller ones, and in consequence weighed less even though their linear measurements may have been as great or even greater. Or it may have been that the bees in the heavier lot had absorbed moisture from the air just prior to, or during, the weighing process. It is concluded, therefore, that dry weight alone would be highly unsatisfactory as an index to the size of the bee.

BEES FROM COLONY 21

Statistics of the various measurements on the bees from colony 21 are recorded in table 4. The trend is much the same as before, with small and erratic differences in dry weight.

One is impressed by the fact that all measurements tend to increase with foundation cell size. As between A and C, most of the differences are sta-

TABLE 3. MEANS, MEAN DIFFERENCES AND STANDARD ERRORS OF MEASUREMENTS OF BEES FROM COLONY 18.

Measurement taken	Size of cell A	Mean diff. of A and B	Size of cell B	Mean diff. of B and C	Size of cell C	Mean diff. of A and C
	M ± δM.	M.D. ± δM.D.	M ± δM.	M.D. ± δM.D.	M ± δM.	M.D. ± δM.D.
Dry weight	16.1020 ±0.2054	−0.7895* ±0.2422	15.3125 ±0.1284	1.6115* ±0.1542	16.9240 ±0.0854	0.8220* ±0.2224
Length of right forewing	9.6390 ±0.0241	−0.0619 ±0.0360	9.5771 ±0.0268	0.1531* ±0.0322	9.7302 ±0.0178	0.0912* ±0.02996
Width of right forewing	3.3210 ±0.0113	0.0094 ±0.0146	3.3304 ±0.0093	0.0140 ±0.0123	3.3444 ±0.0080	0.0234 ±0.0138
Sum of widths of third and fourth tergites	4.9212 ±0.0187	0.0063 ±0.0260	4.9275 ±0.0181	0.0605* ±0.0276	4.9880 ±0.0208	0.0668* ±0.0280
Length of proboscis	6.5778 ±0.0166	0.0130 ±0.0314	6.5908 ±0.0266	0.0794* ±0.0305	6.6702 ±0.0150	0.0924* ±0.0224

*Mean difference is statistically significant.

TABLE 4. MEANS, MEAN DIFFERENCES AND STANDARD ERRORS OF MEASUREMENTS OF BEES FROM COLONY 21.

Measurement taken	Size of cell A	Mean diff. of A and B	Size of cell B	Mean diff. of B and C	Size of cell C	Mean diff. of A and C
	$M \pm \delta M.$	$M.D. \pm \delta M.D.$	$M \pm \delta M.$	$M.D. \pm \delta M.D.$	$M \pm \delta M.$	$M.D. \pm \delta M.D.$
Dry weight	15.7350 ±0.1654	−0.2445 ±0.2694	15.4907 ±0.2127	0.4449 ±0.2456	15.9356 ±0.1228	0.2006 ±0.2060
Length of proboscis	6.6710 ±0.0183	0.0741* ±0.0235	6.7451 ±0.0148	0.0269 ±0.0195	6.7720 ±0.0127	0.1010* ±0.0227
Length of right forewing	9.6930 ±0.0227	0.1442* ±0.0290	9.8372 ±0.0181	0.0657 ±0.0333	9.9029 ±0.0280	0.2099* ±0.0360
Width of right forewing	3.3093 ±0.0133	0.0474* ±0.0155	3.3567 ±0.0079	−0.0003 ±0.0146	3.3564 ±0.0123	0.0471* ±0.0181
Sum of widths of third and fourth tergites	4.9938 ±0.0169	0.1611* ±0.0282	5.1549 ±0.0226	0.0253 ±0.0282	5.1802 ±0.0169	0.1864* ±0.0239

*Mean difference is statistically significant.

tistically significant, but between A and B as well as between B and C the differences are smaller, in a number of instances being either negative or non-significant.

CORRELATIONS AMONG MEASUREMENTS OF WORKER BEES FROM THREE SIZES OF BROOD CELLS

In tables 5, 6 and 7 are presented the zero order correlation coefficients among measurements taken on parts of the worker bees from each size of foundation cell. These correlations tend to be positive, the only negatives being non-significant. Roughly half the positive correlations are significant. Since we shall present below multiple regressions based on these correlations, we shall defer discussions of the details.

VARIATION IN MEASUREMENTS OF WORKER BEES FROM COLONY 25

In table 8 may be found the standard deviations of eight measurements of worker bees taken from three sizes of foundation cells in colony 25. It is clear that there is no consistent increase in variation with increasing size of cell. In fact, a majority of the characters have smaller standard deviations in the larger cells. Only in dry weight is there a highly significant increase in variation associated with increasing cell size; its standard deviation in C is notably larger than in A and B. But as pointed out above, dry weight is difficult to measure accurately, so that variations in it are unreliable. With this one exception, our findings are in contrast with those of Michailov (25), who concluded that worker bees reared in drone cells are more variable in their measurements than their worker cell sisters.

TABLE 5. CORRELATION COEFFICIENTS OF MEASUREMENTS OF BEES FROM COLONY 25.

Measurement taken	Size of cell	Length of right fore-wing	Width of of right fore-wing	Sum of widths of III & IV tergites	Length of pro-boscis	Length of glossa	Length of mentum	Sum of lengths of mentum and glossa
Dry weight	A	+0.4738*	+0.4255*	+0.3873*	+0.4460*	+0.3410†	+0.3135†	+0.4227*
	B	+0.3217*	+0.3780*	+0.2242	+0.0919	−0.0483	+0.0378	+0.0191
	C	+0.3135*	+0.2674	+0.3354†	+0.1939	−0.0591	+0.2634	+0.0596
Length of right forewing	A		+0.7095*	+0.3011†	+0.7274*	+0.6902*	+0.3538†	+0.7404*
	B		+0.4427*	+0.2108	+0.2818	+0.2934†	+0.2258	+0.3582†
	C		+0.6312*	+0.3635†	+0.6297*	+0.5216*	+0.3324†	+0.6086*
Width of right forewing	A			+0.2444	+0.4716*	+0.3713*	+0.4060*	+0.4887*
	B			+0.1164	+0.1776	+0.0861	+0.3314†	+0.2371
	C			+0.1831	+0.2918	+0.3113*	+0.1121	+0.3264†
Sum of widths of third and fourth tergites	A				+0.2120	+0.2559	−0.0886	+0.1991
	B				−0.1406	−0.0476	−0.0733	−0.0764
	C				+0.2241	+0.1900	+0.2124	+0.2607
Length of proboscis	A					+0.3977*	+0.9091*	+0.9547*
	B					+0.4967*	+0.8487*	+0.9546*
	C					+0.5570*	+0.7881*	+0.9430*
Length of glossa	A						+0.0682	+0.4311*
	B						+0.0288	+0.5219*
	C						+0.1835	+0.3121†
Length of mentum	A							+0.6565*
	B							+0.8838*
	C							+0.8355*

*Correlation coefficient is highly significant.
†Correlation coefficient is significant but not highly so.

TABLE 6. CORRELATION COEFFICIENTS OF MEASUREMENTS OF BEES FROM COLONY 21.

Measurement taken	Size of cell	Length of right fore-wing	Width of right fore-wing	Sum of widths of III and IV tergites	Length of proboscis
Dry weight	A	+0.3346**	+0.2355	+0.4928*	+0.1443
	B	+0.1602	+0.2519	+0.1342	+0.4181*
	C	+0.4966*	+0.2603	+0.6191*	+0.3085*
Length of right forewing	A		+0.4503*	+0.2732	−0.0064
	B		+0.6184*	+0.4551*	+0.1927
	C		+0.6522*	+0.5483*	+0.4274*
Width of right forewing	A			+0.2032	+0.3257**
	B			+0.1493	+0.0879
	C			+0.3365**	+0.4068*
Sum of widths of third and fourth tergites	A				+0.1308
	B				−0.0186
	C				+0.4624*

*Correlation coefficient is highly significant.
**Correlation coefficient is significant but not highly so.

MULTIPLE REGRESSION STUDIES

The multiple regressions of table 8 were computed in an effort to isolate body measurements, other than the proboscis, which might be used as indicators of proboscis length. The results

TABLE 7. CORRELATION COEFFICIENTS OF MEASUREMENTS OF BEES FROM COLONY 18.

Measurement taken	Size of cell	Length of right fore-wing	Width of right fore-wing	Sum of widths of III and IV tergites	Length of proboscis
Dry weight	A	+0.0817	−0.2170	+0.2613	+0.0074
	B	+0.5017*	+0.2876	+0.5345*	+0.2790
	C	+0.3704*	+0.0238	+0.3418**	−0.0058
Length of right forewing	A		+0.3734**	−0.0895	+0.0744
	B		+0.3839*	+0.1112	+0.3168**
	C		+0.3703*	−0.1247	+0.2295
Width of right forewing	A			−0.1013	+0.4816*
	B			+0.1779	+0.2810
	C			−0.2639	+0.6135*
Sum of widths of third and fourth tergites	A				+0.2492
	B				+0.1788
	C				+0.2699

*Correlation coefficient is highly significant.
**Correlation coefficient is significant but not highly so.

TABLE 8. STATISTICS OF MEASUREMENTS OF WORKER BEES FROM COLONY 25.

1. Standard Deviations

Character measured	Symbol	Size of cell A	Size of cell B	Size of cell C
Dry weight, mg.	D	0.6228	0.7372	1.8502
Length of right forewing, mm.	L	0.1513	0.1062	0.1173
Width of right forewing, mm.	W	0.0721	0.0616	0.0530
Sum of widths of third and fourth tergites, mm.	S	0.0985	0.1144	0.1109
Length of proboscis, mm.	X	0.1263	0.0807	0.0982
Length of mentum, mm.	M	0.0408	0.0366	0.0395
Length of glossa, mm.	G	0.0978	0.0616	0.0828

2. Standard regression coefficients and multiple correlation coefficient.

	A	B	C
$\beta \times D \cdot LWS$	0.1587	0.0216	0.0145
$\beta \times L \cdot DWS$	0.7481*	0.2910**	0.7448*
$\beta \times W \cdot LDS$	−0.1154	0.0657	−0.1788
$\beta \times S \cdot LWD$	−0.0464	−0.2144	−0.0187
$R \times . DLWS$	0.7422*	0.3540	0.6445*

*Correlation coefficient is highly significant.
**Correlation coefficient is significant but not highly so.

show that the only character with significant regression coefficients was the length of right forewing (L). Let us compare its zero order correlation coefficients (table 5) with the values of R in the three sizes of cells:

Size of cell	A	B	C
Zero order correlation	0.7274	0.2818	0.6297
Multiple correlation	0.7422	0.3540	0.6445

TABLE 9. ZERO ORDER AND MULTIPLE CORRELATION COEFFICIENTS AMONG LENGTH OF RIGHT FOREWING (L), LENGTH OF MENTUM (M) AND PROBOSCIS LENGTH (X). (BEES FROM COLONY 25.)

Correlation	A	B	C
rLX	0.7274	0.2818	0.6297
rMX	0.9090	0.8487	0.7881
RX·LM	0.9999	0.8538	0.8793

Regressions of X on L and M

Size of Cell	Diameter* mm.	Regression
A	4.24	$\overline{X} = 0.3872L + 2.306M - 1.159$
B	4.49	$\overline{X} = 0.0722L + 1.824M - 2.720$
C	4.67	$\overline{X} = 0.3461L + 1.618M + 0.481$

*This is the distance between opposite vertices.

It is clear that, for practical purposes, length of right forewing alone contributes all the available information for estimating length of proboscis from body measurements exclusive of the proboscis. The other three characters might as well be ignored. Measurements of the length of right forewing offer real advantages for estimating proboscis length, because the technique required is very simple, and the measurements can be obtained with a fraction of the work involved in securing measurements of the proboscis.

The question then arises as to whether the measurement of some one part of the proboscis might be even a better index to total proboscis length. Reference to table 5 shows that mentum length alone is more highly indicative of proboscis length than is any other single measurement upon which data were taken. While the mentum can be measured with far less difficulty than the entire proboscis, it is not so easy as the wing. Hence for preliminary surveys much valuable time could be saved by using length of right forewing as an indicator of proboscis length, but for more precise investigations mentum length should be used.

Would it be worthwhile to measure both right forewing and mentum in order to attain precision of estimation of proboscis length? For contrast, the zero order and multiple correlation coefficients are set down together in table 9. The increase in precision attained by using both measurements is not great but might be well worth the extra trouble if critical decisions are to be made.

Those who wish to compare their own data with those in this report will find in table 9 the regressions of proboscis length on wing length and mentum length. These may be used in estimating the length (millimeters) of proboscis if appropriate measurements on the cells and worker bees are available. The regression for cell size A is the one that would apply to bees reared in combs built on ordinary commercial foundation.

DISCUSSION

The data presented show conclusively that size of brood cell is a factor in determining the size of the adult worker bee and that significantly larger bees are obtained through the use of artificial foundation having enlarged cell bases.

These data, therefore, substantiate the contentions of Baudoux (6) and Pincot, according to Gillette-Croix (13), that worker bees reared in brood combs constructed from enlarged cell foundation are larger than their worker cell sisters. However, we cannot agree with Baudoux either in the magnitude of the results he obtained or the consistency of them. While Baudoux records an increase of from 11.9 percent to 25 percent in tongue reach as the size of brood cell increases from 850 cells per square decimeter to 700 cells per square decimeter, we are able to record increases of only 2.07 percent, 1.51 percent and 1.40 percent in length of proboscis for colonies 25, 21 and 18 respectively.

These data, however, compare favorably with an increase of 4.82 percent obtained by Michailov (25) in measuring the probocides of worker bees reared in drone cells as compared with worker bees reared in normal worker cells. They also compare favorably with results obtained on worker bees reared in new combs and worker bees reared in old combs. Here Michailov (26) records an increase of 1.05 percent in length of proboscis for those reared in new combs.

Our data from colony 25 substantiate those of Michailov (25 and 26) which show that an increase in the size of brood cells is accompanied by a corresponding increase in the weight, length of right forewing, width of right forewing, sum of widths of third and fourth tergites and length of proboscis. Colonies 18 and 21 yielded somewhat conflicting results.

Whether the increases in the measurements of the worker bees recorded in these data are significantly related to honey production has yet to be proved.[5]

It is apparent, however, that size of brood cells alone is not sufficient to produce a much larger worker bee. It is reasonable to state that selection and breeding of bees plus the appli-

[5] See footnote 3, page 261.

cation of extrinsic factors such as size of brood cell should accomplish marked results in that direction and that, with selection and breeding for a larger bee, a larger brood cell may be a necessary factor.

It is of interest to mention that difficulties were encountered in getting the queens to oviposit worker eggs in the enlarged cells when all three sizes were in the same hive at the same time. This was particularly true in the case of size of cell C. While the worker bees apparently recognized no difference in constructing the three sizes of cells, the queen bees showed a preference for the normal-sized cells for ovipositing. This observation agrees with experiments conducted by Lovchinovskaya (22).

From brood counts made on colonies supplied with brood combs constructed from foundation having the same size of cells, it was shown that the reaction of both queens and worker bees to each size of cell was apparently the same. This observation corroborates the experiments of Lovchinovskaya (22) and Baudoux (6). Further study of the brood rearing activities of colonies supplied with combs containing enlarged brood cells should be made throughout a period of two or more seasons.[6]

When the data of this experiment were being calculated, Götze (14) stated that a judgment of the relative length of proboscis was obtained from the measurement of the second member of the right labial palpus, and a formula was prescribed for estimating length of proboscis from it. Consequently a study was made of these measurements on bees from colony 14. From a study of the arithmetic means and correlation coefficients, it was found that the second member of the right labial palpus is not correlated with length of proboscis.

[6] Practical experience with brood combs constructed from enlarged cell foundation has shown that there is a tendency for the C size to contain the most drone brood as the age of the combs increases.

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