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Original article

Quantitative analysis of intracolonial and intercolonial morphometric variance in honeybees, *Apis mellifera* and *Apis cerana*

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Abstract – Systematic analyses were used to test the significance of colony and individual bee sample sizes to studies of honeybee populations from six geographically distant and distinct populations of *Apis mellifera* and *Apis cerana*. All possible combinations of sampling *k* colonies from a total number of *n* colonies at each locality were generated. The effects of colony sample size and individual sample size per colony on the variation in the mean values of nine morphological characters are shown (1) separately and (2) together. The percentage increase or decrease of the maximum and minimum mean values from the estimated population means are calculated for all possible combinations of *k* colonies sampled from the total number of *n* colonies at each locality. Samples of 5 or 6 colonies with 10 bees per colony per locality are shown to be adequate for characters related to size and wing venation and adequate for pigmentation characters only when the bees are homogeneous in colour.

colony sample size / individual bee sample size / intercolonial and intracolonial variability / morphometric character / *Apis mellifera* / *Apis cerana*

1. INTRODUCTION

It is characteristic of honeybees that the mean variability of morphological characters in a single colony is less than that of an apiary of many colonies (Alpatov, 1929). Moreover, colonies differ not only in the averages of their characteristics but also in the coefficients of correlation between characteristics (Alpatov and Tjunin, 1925). Because intracolonial and intercolonial differences in populations vary, the Russian school assiduously examined large samples in their studies of Eurasian honeybees early in the last century (Alpatov, 1929). Interestingly, although the fundamental principles of quantitative genetics had been established contemporaneously (Fisher, 1930; Wright, 1968, 1969), their application to population studies of honeybees was necessarily precluded until the polyandrous nature of mating in honeybees was first documented (Roberts, 1944).

Recognition of polyandry in queen honeybees provided a means for linking the theoretical basis of intracolonial and intercolonial variation to quantitative genetics. This is because the sources of variation, in a genetic sense, derive from the fact that a honeybee colony is a collection of different families of full and half-sisters that form from separate patrilines (Moritz and Southwick, 1992).

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Thus, in the case of quantitative traits of high heritability such as morphological characters (Oldroyd and Moran, 1983; Oldroyd et al., 1991), intracolonial (= "within colony") variation may be greater than intercolonial (= "among colony") variation in different populations of honeybees (Adams et al., 1977; Oldroyd and Moran, 1983; Rinderer et al., 1990); but, the obverse occurs in several natural populations of honeybees (Hepburn and Radloff, 1998)

Were intracolonial variance the greater for a particular apiary/population and given a fixed "within colony" sample size, then just a few colonies should suffice as a measure of variation for the larger population. Conversely, the smaller the "within colony" sample, the greater the "among colony" samples required (Falconer, 1989). Consequently, there are different possible optimal solutions for sampling, which will vary with different traits, numbers of individuals per colony and number of colonies. A more difficult hurdle is the comparison of different populations using different numbers of bees within and between colonies. Here the solution is not obvious because the evolutionary factors leading to divergence in populations cannot be precisely defined.

Thus, it remains a very curious anomaly that the significance of sample size within and among colonies to the measurement of intracolonial and intercolonial variance along a continuum in honeybee populations has not previously been quantitatively determined with rigourous statistical procedures. Thus, taking leaves from both the Alpatov and Fisher-Wright traditions, we report the results of systematic analyses of the statistical significance of colony sample size and individual bee sample size to studies of honeybee populations from several geographically distant and distinct populations of *Apis mellifera* and *A. cerana*. Specifically, we examine several relationships (1) the nature of average colony variation compared to that of an "apiary" (any concentration of honeybee colonies close by each other), (2) the effect of colony sample size on the means and standard deviations of the morphometric characters, (3) the effect of individual bee sample size on the means and standard deviations of the morphometric characters, and (4) the resolution of intracolonial and intercolonial variance on population mean estimates of the morphometric characters.

2. MATERIALS AND METHODS

Measurements of nine morphometric characters, (Ruttner (1988) numbers given in brackets) namely size: length of cover hair on tergite 5 (1), width and length of wax plate on sternite 3 (11) and (13); angles of wing venation: B4 (22), N23 (30), O26 (31); pigmentation: abdominal tergite 2 (32), scutellum (35) and scutellar plate (36) of *A. cerana* worker honeybees from Kathmandu, Nepal; *A. mellifera* worker honeybees from Beaufort West and Queenstown, South Africa; Njiro, Tanzania; Ngong Hills, Kenya and Assiut, Egypt were used in the analysis. Colony means, intercolonial and intracolonial standard deviations and coefficients of variation were calculated for the total apiary at each locality.

A computer programme was written in C to generate all possible combinations of k colonies sampled from the total number of n colonies at each locality. Samples of 5 and 8 were taken from Beaufort West colonies ($n = 10$) and Queenstown colonies ($n = 11$) in South Africa; samples of 5 and 10 were taken from Ngong Hills colonies (n = 13) in Kenya and Assiut colonies ($n = 15$) in Egypt and samples of 5, 10 and 15 were taken from Njiro colonies ($n = 20$) in Tanzania and Kathmandu colonies ($n = 21$) in Nepal. The number of colonies, the number of worker honeybees sampled from each colony and the number of combinations generated are shown in Table I.

Contour surface plots were used to evaluate the effect of both colony sample size and individual bee sample size together on the mean values of the morphometric characters. A surface was fitted to the XYZ coordinate data according to the distance weighted least squares smoothing procedure which is particularly useful in uncovering complex non-linear relationships between the two variables, namely colony and individual bee sample sizes (Statistica, 1995).

3. RESULTS

3.1. Ratio of average colony coefficients of variation to total variation of the whole apiary

The results show that the percentage ratios for the average colony coefficients of variation varied with the different morphometric characters considered (Tab. II). The low ratios for

Table I. Localities where worker honeybees were sampled, number of colonies (*n*), number of individual bees and number of combinations generated sampling *k* colonies from *n* colonies.

Locality	Coordinates	No. of colonies (n)	No. of bees/colony	No. of combinations k = 5	k = 8	k = 10	k = 15
Beaufort West	32.18S, 22.36E	10	20	252	45	-	-
Queenstown	31.52S, 27.00E	11	20	462	165	-	-
Njiro	03.23S, 36.40E	20	15	15504	-	184756	15504
Ngong Hills	01.24S, 36.38E	13	15	1287	-	286	-
Assiut	27.14N, 31.07E	15	20	3003	-	3003	-
Kathmandu	27.45N, 85.22E	21	20	20349	-	352716	54264

Table II. Percentage ratios of mean colony coefficient of variation to total variation of the whole apiary for nine morphometric characters.

Locality	Morphometric characters								
		size		wing venation			pigmentation		
	(1)	(11)	(13)	(22)	(30)	(31)	(32)	(35)	(36)
Beaufort West	82.2	94.3	89.5	87.1	80.6	79.1	89.6	90.4	115.7
Queenstown	92.0	81.8	87.7	88.0	78.2	89.1	88.5	98.5	93.5
Njiro	80.2	67.8	60.0	93.4	73.7	91.1	66.8	87.4	97.6
Ngong Hills	86.8	82.2	85.0	93.7	80.2	96.4	101.2	100.4	80.0
Assiut	53.2	55.1	59.5	79.2	89.0	79.0	36.0	41.3	60.4
Kathmandu	80.3	nm	87.6	83.6	nm	nm	84.3	73.5	88.1

nm = character not measured.

Assiut bees indicate the high intercolonial heterogeneity of the bees for morphometric characters related to size and pigmentation. Closer investigation of the 15 colonies from Assiut revealed 3 colonies with larger and darker bees (with low intracolonial variation) than bees from the other 12 colonies, which were smaller and lighter in colour (with low intracolonial variation). This gave a low average colony coefficient of variation compared to the high intercolonial variation of the whole apiary. Ratios greater than 100% [Ngong Hills (32) and Beaufort West (36)] indicate high average colony coefficients of variation resulting from bees with mixed pigmentation within the same colonies (i.e. high intracolonial variation).

3.2. The effect of colony sample size on the mean values of morphometric characters

Table III shows the maximum and minimum mean values together with intercolonial standard deviations of three morphometric characters [size: (13), wing angle: (22) and pigmentation: (32)] when samples of all combinations of *k* colonies from *n* possible colonies from each locality are generated. The results show that the variation in the mean values of the characters increases as the number of colonies sampled decreases (Tab. III). The effect of the colony sample size on the mean values is seen from the percentage increase or decrease of the maximum and minimum mean values from the estimated population mean. As *k* decreases the absolute percentages increase. The size of this effect varies according to the character considered and also the degree of homogeneity or heterogeneity of the honeybees within and between colonies from individual localities. *Note that the maximum and minimum mean values are the extreme scenarios. When k colonies are sampled at random the probability of selecting colonies that will result in one of these extreme cases is very small (i.e. Assiut: 1/3003 = 0.00033, Queenstown: 1/462 = 0.0022).* For example, random samples of 5 colonies (with 20 bees per colony) at Assiut and Queenstown gave

Table III. Maximum and minimum mean values together with intercolonial standard deviations (sd) of three morphometric characters when samples of all combinations of *k* colonies from *n* possible colonies from each locality are generated. Means ± intercolonial standard deviations are also given when random samples of *k* colonies taken.

Morphometric character (13)

Locality		Mean	Max	Min k = 5	Sample mean	Max	Min k = 8	Sample mean	Max	Min k = 10	Sample mean	Max	Min k = 15	Sample mean
Beaufort West	$\bar{X}$	2.1686	2.2039	2.1329	2.1738	2.1798	2.1534	2.1728	-	-	-	-	-	-
	sd	(0.0428)	(0.0289)	(0.0110)	(0.0365)	(0.0398)	(0.0307)	(0.0461)	-	-	-	-	-	-
	%↑↓		1.63%	–1.64%	0.24%	0.52%	–0.70%	0.19%	-	-	-	-	-	-
Queenstown	$\bar{X}$	2.1653	2.1954	2.1356	2.1566	2.1821	2.1526	2.1547	-	-	-	-	-	-
	sd	(0.0368)	(0.0073)	(0.0329)	(0.0487)	(0.0193)	(0.0351)	(0.0383)	-	-	-	-	-	-
	%↑↓		1.39%	–1.37%	–0.40%	0.78%	–0.59%	–0.49%	-	-	-	-	-	-
Njiro	$\bar{X}$	2.1476	2.2536	2.0396	2.1529	-	-	-	2.2185	2.0766	2.1338	2.1835	2.1122	2.1476
	sd	(0.0851)	(0.0242)	(0.0160)	(0.0934)	-	-	-	(0.0437)	(0.0465)	(0.0966)	(0.0647)	(0.0655)	(0.0927)
	%↑↓		4.94%	–5.02%	0.25%	-	-	-	3.31%	–3.30%	–0.64%	1.68%	–1.64%	0.00%
Ngong Hills	$\bar{X}$	2.1086	2.1470	2.0753	2.1065	-	-	-	2.1199	2.0888	2.1121	-	-	-
	sd	(0.0404)	(0.0449)	(0.0135)	(0.0427)	-	-	-	(0.0403)	(0.0162)	(0.0461)	-	-	-
	%↑↓		1.82%	–1.58%	–0.10%	-	-	-	0.54%	–0.94%	0.16%			
Assiut	$\bar{X}$	2.0370	2.1624	1.9670	2.0303	-	-	-	2.0875	1.9902	2.0206	-	-	-
	sd	(0.1172)	(0.1133)	(0.0322)	(0.0927)	-	-	-	(0.1293)	(0.0504)	(0.1258)	-	-	-
	%↑↓		5.63%	–3.92%	–0.33%	-	-	-	1.97%	–2.79%	–0.80%	-	-	-
Kathmandu	$\bar{X}$	1.9613	2.0032	1.9224	1.9572	-	-	-	1.9898	1.9330	1.9784	1.9759	1.9457	1.9605
	sd	(0.0326)	(0.0186)	(0.0097)	(0.0286)	-	-	-	(0.0307)	(0.0293)	(0.0322)	(0.0275)	(0.0357)	(0.0290)
	%↑↓		2.13%	–1.98%	–0.21%	-	-	-	1.45%	–1.44%	0.87%	0.74%	–0.80%	–0.04%

Morphometric character (22)

Locality		Mean	Max	Min k = 5	Sample mean	Max	Min k = 8	Sample mean	Max	Min k = 10	Sample mean	Max	Min k = 15	Sample mean
Beaufort West	$\overline{X}$	101.6985	103.9290	99.4900	101.7000	102.4280	100.6230	101.5283	-	-	-	-	-	-
	sd	(2.7938)	(2.1391)	(0.8134)	(5.8715)	(2.6455)	(1.8706)	(2.6279)	-	-	-	-	-	-
	%↑↓		2.19%	–2.17%	0.00%	0.72%	–1.06%	–0.17%	-	-	-	-	-	-
Queenstown	$\overline{X}$	102.3333	104.1210	100.7400	103.6061	103.0750	101.3080	102.3836	-	-	-	-	-	-
	sd	(2.1295)	(1.9053)	(0.6035)	(2.2537)	(2.0313)	(1.1167)	(2.4026)	-	-	-	-	-	-
	%↑↓		1.75%	–1.56%	1.24%	0.72%	–1.00%	0.05%	-	-	-	-	-	-
Njiro	$\overline{X}$	106.1390	109.0580	104.2030	106.5415	-	-	-	107.6090	104.6680	105.4230	106.7840	105.1650	106.0765
	sd	(1.9916)	(1.1693)	(0.5729)	(2.8530)	-	-	-	(1.7706)	(0.6593)	(1.8913)	(1.8720)	(0.9656)	(1.9396)
	%↑↓		2.75%	–1.82%	0.38%	-	-	-	1.38%	–1.39%	–0.67%	0.61%	–0.92%	–0.06%
Ngong Hills	$\overline{X}$	102.8636	104.9260	100.6020	104.2244	-	-	-	103.7490	101.9990	102.8247	-	-	-
	sd	(2.2532)	(1.5525)	(0.9623)	(2.2571)	-	-	-	(1.7296)	(1.6309)	(2.5583)	-	-	-
	%↑↓		2.00%	–2.20%	1.32%	-	-	-	0.86%	–0.84%	–0.04%	-	-	-
Assiut	$\overline{X}$	101.8182	105.8700	98.0900	101.3700	-	-	-	103.7110	99.7614	101.5477	-	-	-
	sd	(3.9856)	(4.0086)	(1.0014)	(4.2950)	-	-	-	(3.5654)	(1.9626)	(4.4984)	-	-	-
	%↑↓		3.98%	–3.66%	–0.44%	-	-	-	1.86%	–2.02%	–0.26%	-	-	-
Kathmandu	$\overline{X}$	107.5154	110.4480	103.8870	110.1005	-	-	-	109.6350	105.3270	107.4664	108.8470	106.4170	107.3158
	sd	(2.6425)	(1.0723)	(3.4912)	(1.4173)	-	-	-	(1.1525)	(1.9557)	(3.1543)	(2.9260)	(2.0079)	(2.6522)
	%↑↓		2.73%	–3.38%	2.40%	-	-	-	1.97%	–2.04%	–0.04%	1.24%	–1.02%	–0.18%

Morphometric character (32)

Locality		Mean	Max	Min k = 5	Sample mean	Max	Min k = 8	Sample mean	Max	Min k = 10	Sample mean	Max	Min k = 15	Sample mean
Beaufort West	$\bar{X}$	6.2525	7.4747	5.0303	5.8100	6.6392	5.8176	5.8291	-	-	-	-	-	-
	sd	(1.3872)	(0.6850)	(0.3445)	(1.2407)	(1.2714)	(1.1624)	(1.2069)	-	-	-	-	-	-
	%↑↓		19.55%	–19.55%	–7.08%	6.19%	–6.96%	–6.77%	-	-	-	-	-	-
Queenstown	$\bar{X}$	7.1142	7.7200	6.4646	7.1010	7.4528	6.7924	7.0629	-	-	-	-	-	-
	sd	(0.7158)	(0.3420)	(0.4156)	(0.7268)	(0.4846)	(0.5484)	(0.7682)	-	-	-	-	-	-
	%↑↓		8.52%	–9.13%	–0.18%	4.76%	–4.52%	–0.72%	-	-	-	-	-	-
Njiro	$\bar{X}$	7.9833	8.9466	6.9600	8.0533	-	-	-	8.6666	7.3000	8.0667	8.3244	7.6622	8.0667
	sd	(0.8261)	(0.0869)	(0.5549)	(0.9011)	-	-	-	(0.3340)	(0.5400)	(0.8219)	(0.5827)	(0.6944)	(0.8648)
	%↑↓		12.07%	–12.82%	0.88%	-	-	-	8.56%	–8.56%	1.04%	4.27%	–4.02%	1.04%
Ngong Hills	$\bar{X}$	6.0205	7.5600	4.4666	6.7466	-	-	-	6.5800	5.4866	6.3666	-	-	-
	sd	(1.5382)	(0.5936)	(0.6649)	(1.0805)	-	-	-	(1.2380)	(1.2942)	(1.3724)	-	-	-
	%↑↓		25.57%	–25.81%	12.06%	-	-	-	9.29%	–8.87%	5.75%	-	-	-
Assiut	$\bar{X}$	7.1074	8.6700	4.5510	6.4900	-	-	-	8.3600	6.3181	7.4800	-	-	-
	sd	(2.5849)	(0.3402)	(3.3275)	(3.0396)	-	-	-	(0.3984)	(2.877)	(2.3317)	-	-	-
	%↑↓		21.99%	–35.97%	–8.69%	-	-	-	17.62%	–11.10%	5.24%	-	-	-
Kathmandu	$\bar{X}$	7.8571	7.9900	7.6600	7.9000	-	-	-	7.9650	7.7450	7.8800	7.9300	7.8067	7.8700
	sd	(0.1316)	(0.1274)	(0.1387)	(0.1458)	-	-	-	(0.1230)	(0.1471)	(0.1358)	(0.0621)	(0.1222)	(0.1236)
	%↑↓		1.69%	–2.51%	0.55%	-	-	-	1.37%	–1.43%	0.29%	0.93%	–0.64%	0.16%

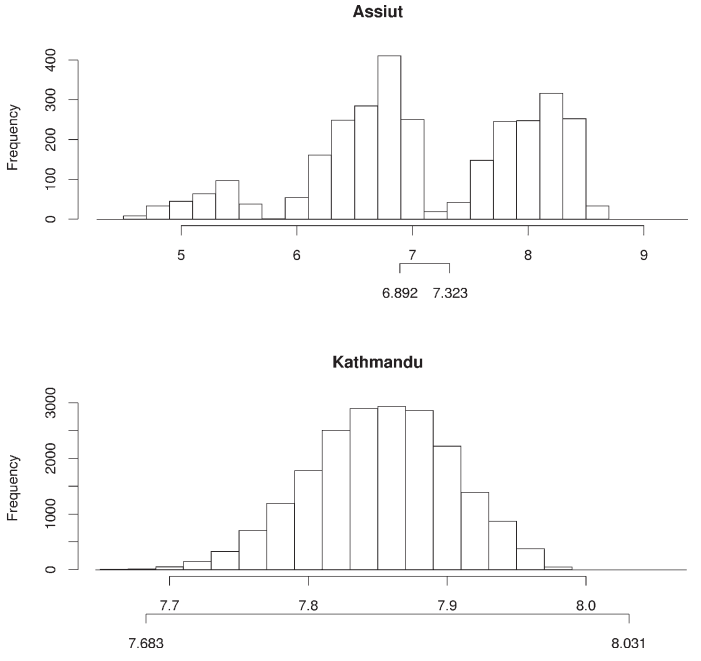

Figure 1. Frequency distribution of all combinations of 5 colony samples of honeybees from Assiut (high intercolonial variance) and Kathmandu (low intercolonial variance) for the pigmentation of abdominal tergite 2 (32).

mean values of 2.030 ± 0.093 and 2.157 ± 0.049, respectively, for the length of wax plate on sternite 3 (13) compared to 2.037 ± 0.117 and 2.165 ± 0.037 when 15 and 11 colonies, respectively, were sampled (Tab. III).

When considering the length of wax plate on sternite 3 (13) the percentage increase or decrease in the maximum and minimum mean values for the heterogeneous Assiut bees increased or decreased from 1.97% to –2.79% when $k = 10$ to 5.63% to –3.92% when $k = 5$ whilst for the homogeneous Queenstown bees there was only an increase or decrease from 0.78% to –0.59% when $k = 8$ to 1.39% to –1.37% when $k = 5$. Similar effects of colony sample size are obtained for the other two morphometric characters related to size, namely length of hair on tergite 5 (1) and width of wax plate (11) on sternite 3.

Less effect on colony sample size was found for angles of wing venation (22), (30) and (31) (Tab. III). The range of the absolute percentage increases or decreases for character (22) was 1.56% to –3.98%; for (30) was 1.70% to –3.98% and for (31) was 3.39% to –7.51% when all combinations of 5 colonies were taken. On the other hand, the effect on colony sample size for pigmentation characters was pronounced for apiaries where the bees are extremely heterogeneous in colour (Tab. III). In particular the tri-modal distribution of means for pigmentation (32) for heterogeneous bees from Assiut shows the effect of selecting 5 colonies (range of percentage increase or decrease is –35.97% to 21.99%) (Fig. 1). There is the possibility that all 5 colonies have dark bees, or all 5 have light bees or a mixed combination of dark and light colonies (Fig. 1).

Estimation of the required colony sample size

The least number of colonies that need to be sampled in order to achieve 90% confidence that the difference between the population mean and the sample mean will lie within an acceptable error, say E, was calculated for each morphometric character at each locality.

The formula used for these calculations is given by

$$n = (1.645\ s_{inter} / E)^2 \qquad [1]$$

where s_{inter} is an estimate of the intercolonial standard deviation when the number of bees in each colony was fixed at the maximum number measured per colony at each locality and E is the size of the error to be tolerated. The estimated colony sample sizes are given in Table IV for characters [size: (13), wing angle: (22) and pigmentation: (32)]. When considering the length of wax plate on sternite 3 (13) the least number of colonies to be sampled, when E = 2.5% of the estimated population mean, is 2 for 4 of the 6 localities with low intercolonial variability (Tab. IV). For the heterogeneous Assiut bees, however, the least number of colonies is 15. Similarly for the wing angle character (22), at least 2 or 3 colonies would need to be sampled from the 5 localities with low intercolonial variability, when E = 2.5% of the estimated population mean, compared to at least 7 colonies at Assiut. The number of required colonies, on the other hand, increases considerably when estimating the population mean of a pigmentation character (32) (Tab. IV). The least number of colonies ranged from 2 colonies (Kathmandu) to 573 colonies (Assiut) when E = 2.5%. If the size of the error to be tolerated is increased to 10% of the estimated population mean then the least number of colonies required at Assiut reduces to 36.

3.3. The effect of individual bee sample size per colony on the mean values of morphometric characters

The effect of using a smaller number of individual bees per colony on the mean values is smaller than the effect of using a smaller number of colonies (Alpatov, 1929). For localities with low intracolonial variability the differences in sampling 5, 10 or 15 bees per colony on the mean pigmentation character (32) differs only in the second decimal place compared to samples of 20 bees per colony. For example, at Assiut where the intracolonial standard deviation is low (s_{intra} = 0.485), the mean values for (32) are 7.106, 7.093, 7.080 when samples of 5, 10 or 15 bees per colony are randomly selected compared to a mean value of 7.107 when all 20 bees are included per colony (absolute percentage increase is 0.38%). On the other hand, at Ngong Hills where the intracolonial variability is high (s_{intra} = 3.185), the differences on the mean values in sampling smaller numbers of bees per colony differs in the first decimal place (absolute percentage difference is 2.99%).

Less effect on individual bee sample size per colony was found for angles of wing venation (22), (30) and (31) and characters related to size (1), (11) and (13). For example, the percentage difference for character (22) at Kathmandu where the intracolonial variability is low (s_{intra} = 3.94) was 0.16% and at Ngong Hills where the intracolonial variability is high (s_{intra} = 6.42) was 0.69%; for (13) was 0.29% (Kathmandu, s_{intra} = 0.061) and was 0.35% (Assiut, s_{intra} = 0.117) when samples of 5 colonies were taken.

Estimation of the required individual bee sample size per colony

The formula used to estimate the least number of bees that need to be sampled per colony in order to achieve 90% confidence that the difference between the population mean and the sample mean will lie within an acceptable error, say E, is given by

$$n = (1.645\ s_{intra} / E)^2 \qquad [2]$$

where s_{intra} is an estimate of the intracolonial standard deviation when the number of colonies was fixed at the maximum number at each locality and E is the size of the error to be tolerated. The estimated individual bee sample sizes per colony are given in Table IV for characters [size: (13), wing angle: (22) and pigmentation: (32)]. For characters related to size (13) and angle of wing venation (22), 10 bees per colony would be more than sufficient for E = 2.5% (Tab. IV). At Assiut where the intracolonial variability in wing angle is higher, the required number of bees increases to at least 20 bees per colony. Again the number of required bees per colony increases considerably when estimating the population mean of a pigmentation character (32) (Tab. IV). The least number of bees per colony, when E = 2.5%, ranged from 11 bees per colony at

Table IV. Inter- and intracolonial standard deviations of the morphometric characters related to size (13), wing angle (22) and pigmentation (32) and estimated colony sample size and number of bees per colony at each locality. E% denotes the percentage error to be tolerated.

Morphometric character (13)

Locality	Mean	Intercolonial sd	Intracolonial sd	E%	No. of colonies	No. of bees
Beaufort West	2.1686	0.0428	0.0810	5%	0.40	1.5
		cv = 1.97	cv = 3.73	2½%	1.69	6.04
Queenstown	2.1653	0.0368	0.0918	5%	0.30	1.9
		cv = 1.70	cv = 4.24	2½%	1.25	7.78
Njiro	2.1475	0.0851	0.0618	5%	1.7	0.9
		cv = 3.96	cv = 2.88	2½%	6.79	3.59
Ngong Hills	2.1087	0.0404	0.0648	5%	0.4	1.02
		cv = 1.92	cv = 3.08	2½%	1.59	3.9
Assiut	2.0472	0.1172	0.1167	5%	3.58	3.55
		cv = 5.75	cv = 5.73	2½%	14.32	14.21
Kathmandu	1.9613	0.0326	0.0611	5%	0.3	1.1
		cv = 1.66	cv = 3.12	2½%	1.2	4.21

Morphometric character (22)

Locality	Mean	Intercolonial sd	Intracolonial sd	E%	No. of colonies	No. of bees
Beaufort West	101.6985	2.7938	4.8104	5%	0.82	2.42
		cv = 2.75	cv = 4.73	2½%	3.27	9.69
Queenstown	102.3333	2.1295	3.9453	5%	0.47	1.61
		cv = 2.08	cv = 3.86	2½%	1.87	6.44
Njiro	106.1385	1.9916	4.9497	5%	0.38	2.33
		cv = 1.88	cv = 4.64	2½%	1.52	9.30
Ngong Hills	102.8635	2.2532	6.4172	5%	0.52	4.21
		cv = 2.19	cv = 6.54	2½%	2.08	16.80
Assiut	101.8182	3.9856	7.0439	5%	1.66	5.18
		cv = 3.91	cv = 6.92	2½%	6.63	20.72
Kathmandu	107.5170	2.6425	3.9397	5%	0.65	1.45
		cv = 2.46	cv = 3.66	2½%	2.62	5.81

Morphometric character (32)

Locality	Mean	Intercolonial sd	Intracolonial sd	E%	No. of colonies	No. of bees
Beaufort West	6.2525	1.3872	2.1027	5%	53.3	122.4
		cv = 22.19	cv = 33.63	2½%	213.13	489.66
Queenstown	7.1142	0.7158	1.6094	5%	10.9	55.4
		cv = 10.06	cv = 22.62	2½%	43.83	221.58
Njiro	7.9833	0.8261	0.9120	5%	11.6	14.1
		cv = 10.35	cv = 11.42	2½%	46.37	56.5
Ngong Hills	6.0205	1.5382	3.1847	5%	70.66	302.89
		cv = 25.55	cv = 52.90	2½%	282.64	1069.4
Assiut	7.1074	2.5849	0.4847	5%	143.2	5.0
		cv = 36.37	cv = 6.82	2½%	572.69	20.14
Kathmandu	7.8571	0.1316	0.3908	5%	0.3	2.7
		cv = 1.68	cv = 4.97	2½%	1.21	10.71

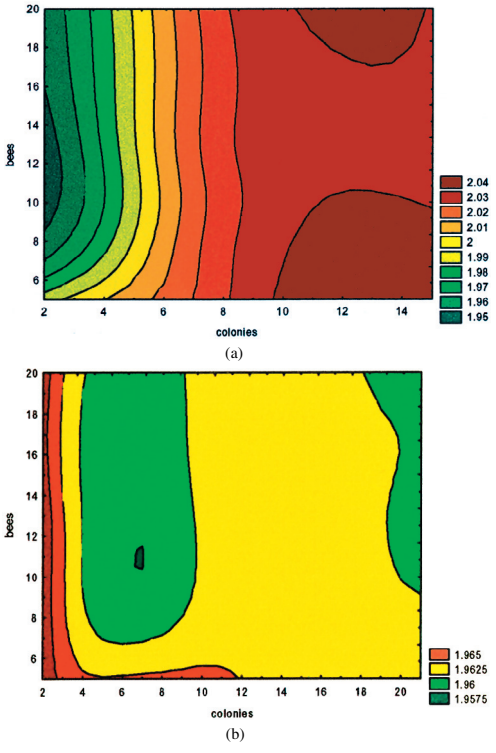

Figure 2. Contour surface plots showing the effect of colony sample size and individual bee sample size on the mean value of the length of wax plate on sternite 3 (13) at (a) Kathmandu and (b) Assiut.

Kathmandu (low intracolonial variability) to 1070 bees per colony at Ngong Hills where the intracolonial pigmentation variability is high. If the size of the error to be tolerated is increased to 10% of the estimated population mean, the estimated number of bees per colony at Ngong Hills reduces to 76.

3.4. The effect of both intercolonial and intracolonial variation on the mean values of morphometric characters

The effect of colony sample size and individual bee sample size per colony on the mean values of the morphometric characters was evaluated by means of contour surface plots. The contour plots confirmed that the effect of using a smaller number of colonies is greater than the effect of using a smaller number of bees per colony. This is indicated by the large differences on the surface along the horizontal plane (i.e. number of colonies) compared to the minor differences along the vertical plane (i.e. number of bees).

Figure 2 shows the contour plot for the length of wax plate on sternite 3 (13) for the homogeneous bees at Kathmandu where both the inter- and intracolonial variances are low. From the plot we see that the estimate of the mean rises rapidly for small numbers of colonies and then stabilizes when only 4 colonies with at least 5 bees per colony are sampled. For the heterogeneous bees from Assiut, on the other hand, we see at least 7 or 8 colonies would need to be sampled. Similar contour plots were obtained for the other characters related to size [(1), (11)] and wing angles [(22), (30), (31)] at the different localities.

Figure 3 shows the contour plot for pigmentation of abdominal tergite 2 (32) for the bees from Assiut where the intercolonial variability is high whilst the intracolonial variability is low. The plot shows the uniformity of the bees within each colony and the large differences between the colonies before stability is reached in the estimate of the mean value when 9 colonies are sampled. The plot for the Kathmandu bees with low inter- and intracolonial variability for (32) indicates that only 5 colonies with at least 8 bees per colony would need to be sampled. Finally figure 3 shows a contour plot for a locality (Ngong Hills) with low intercolonial and yet high intracolonial variances. At least 6 colonies with 10 bees per colony should be sampled in this case.

4. DISCUSSION

The publication of Alpatov (1929) remains a milestone in honeybee morphometrics. Given that he and his statistician colleagues (the renowned Pearson at King's College, Cambridge and Pearl at Cornell University) worked before electrical (much less electronic) calculators were commercially available, it is remarkable to consider the breadth and depth of his analyses. Alpatov (1929) found that the average colony coefficients of variation, expressed as a percentage of the coefficients of variation for a single morphometric character, the length of the honeybee

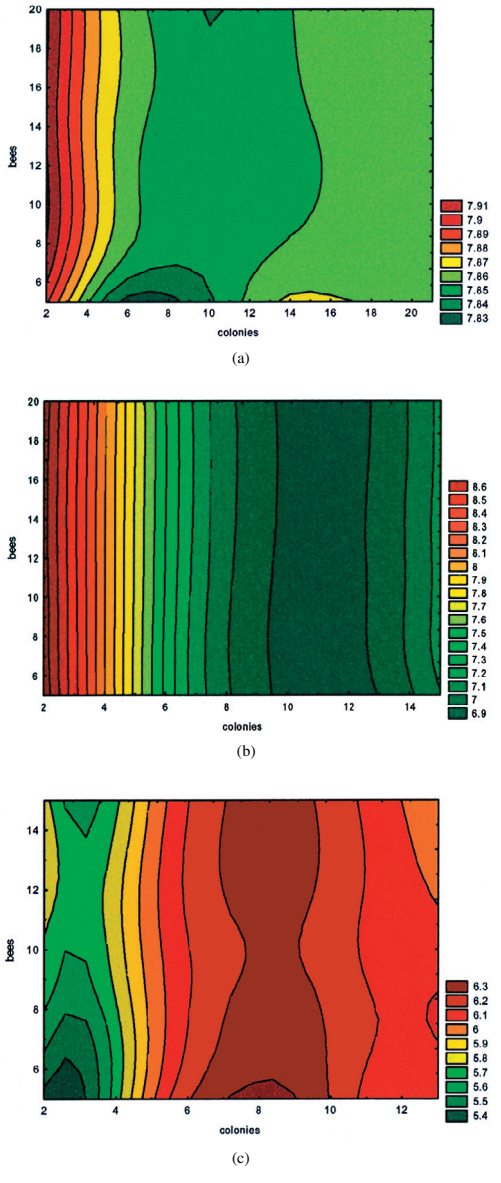

Figure 3. Contour surface plots showing the effect of colony sample size and individual bee sample size on the mean value of the pigmentation of abdominal tergite 2 (32) at (a) Kathmandu, (b) Assiut, (c) Ngong Hills.

proboscis, of the whole apiary for seven apiaries were between 70%–95%. The data obtained from the analysis of nine different morphological characters for size, wing venation, and pigmentation in the present study show that the percentage ratios obtained for six different localities ranged between 36%–116% for the entire suite of characters (Tab. II).

These quite considerably larger variations confirm the general conclusions of Alpatov (1929) as to intrinsic differences in character variation and also indicate that some features are far more conserved than others. Indeed, the nature and extent of variation in morphological characters are empirically evidenced by both their heritability values (Moritz and Klepsch, 1985; Cornuet and Garnery, 1991; Oldroyd et al., 1991) as well as the component effects of environmental modification of the phenotype (Radloff et al., 2002). For the latter, it has recently been demonstrated for all 36 Ruttner (1988) morphometric characters that the pooled, rank order for the coefficients of environmental variation ranged from 0.0157 to 1.0045. Of these, the means and standard deviations of coefficients of environmental variation for characters of size were 0.0374 ± 0.0461, wing venation 0.0478 ± 0.0245 and pigmentation 0.3462 ± 0.3989 (Radloff et al., 2002).

Alpatov (1929) further investigated the effect on the mean proboscis length of bees when sampling 3 colonies at random from a possible 10 colonies of bees at the Tula Apicultural Experiment Station (about 150 km due south of Moscow). Out of a total of 120 possible combinations of 3 colonies, he considered 6 combinations and found the average proboscis length differed from the true mean length of the bees from all 10 colonies in the first decimal place. The range of the percentage increase or decrease from the true mean tongue length was –1.57% to +1.85%. Using a smaller number of colonies, Alpatov (1929) also showed that the difference in the length of the proboscis was larger than the difference using a smaller number of individual bees from all 10 colonies. It was simply not feasible for Alpatov to have considered all combinations of samples of 3 without contemporary computer facilities.

The results of the analysis using nine morphological characters and all possible combinations of k colonies sampled from a total of n colonies from individual localities show that the effect of colony sample size on the mean character values differs according to the

characters and the homogeneity/heterogeneity of the bees within and between colonies. The effect of colony sample size on wing venation and size related characters when samples of 5 colonies are taken is minimal. The more heterogeneous the bees are in colour, the greater the effect of colony sample size on pigmentation characters (32), (35) and (36) when small samples of colonies are analysed: samples of 5 or 6 colonies were adequate for apiaries at Beaufort West, Queenstown and Ngong Hills where the bees are homogeneous in colour, whilst samples of even 10 colonies proved inadequate for the extremely heterogeneous Assiut bees.

Similarly, the data of Table IV unequivocally demonstrate that there is a high degree of phenotypic stability (measured as coefficient of variation for a character) for morphological characters related to size and wing venation. In general, for such characters the intercolonial coefficients of variation are lower than intracolonial values. The same trend occurs for characters of pigmentation but there is a three to four-fold order of magnitude increase in the numbers of individual bees required.

Our results provide a statistical basis for and confirmation of the hypothesis of Alpatov (1929): the number of colonies sampled affects the variation in the mean character values more than the number of bees per colony sampled. However, characters related to size and wing venation are considerably less affected than those of pigmentation so that samples of 5 or 6 colonies per locality with 10 bees per colony have shown to be adequate in the analysis of morphological data in studies of honeybee populations. Pigmentation characters should be included in the analysis with caution unless the bees are known to be homogeneous in colour.

Résumé – Analyses quantitatives de la variance morphométrique intra- et inter-colonies chez les abeilles domestiques *Apis mellifera* et *Apis cerana*. On a effectué des analyses systématiques pour déterminer l'incidence du nombre de colonies échantillonnées et, au sein de chaque colonie, du nombre d'individus analysés, lors d'une étude de six populations distinctes et géographiquement distantes d'*Apis mellifera* et d'*Apis cerana*. Les paramètres utilisés pour ces analyses étaient les moyennes des colonies, les écarts-types inter- et intra-colonies et les coefficients de variation de neuf caractères morphologiques se rapportant à la taille, la veination alaire et la pigmentation. Le quotient en pourcentage des coefficients moyen d'une colonie à la variation totale du rucher pour chacune des six localités a varié de 36 % à 116 % (Tab. II). Les résultats montrent que les quotients varient selon le caractère morphométrique considéré et selon l'homogénéité ou l'hétérogénéité des abeilles au sein de et entre les colonies aux différentes localités.
On a généré toutes les combinaisons possibles d'échantillonnage de *k* colonies à partir de *n* colonies dans chaque localité afin de tester les effets de la taille de l'échantillon de colonies sur la variation des valeurs moyennes des neuf caractères morphométriques. Le pourcentage d'augmentation ou de diminution des valeurs moyennes minimales et maximales de la population estimée montre que la variation des valeurs moyennes des caractères augmente quand le nombre de colonies échantillonnées *k* décroît (Tab. III). Plus les abeilles sont hétérogènes en couleur et plus grand est l'effet de la taille de l'échantillon des colonies sur les caractères de pigmentation lorsqu'on analyse de petits échantillons de colonies (Tab. III, Fig. 1). De même, les données du tableau IV démontrent sans ambiguïté qu'il existe un fort degré de stabilité phénotypique (mesurée par le coefficient de variation d'un caractère) pour les caractères morphométriques liés à la taille et à la veination alaire. En général, pour de tels caractères, les coefficients de variation entre colonies sont plus faibles que les coefficients de variation intra-colonies. La même tendance existe pour les caractères de pigmentation, mais il faut analyser trois à quatre fois plus d'abeilles.
En conclusion, des échantillons de cinq ou six colonies par localité avec 10 abeilles par colonie sont adéquats pour les caractères liés à la taille et à la veination alaire, ainsi que pour les caractères de pigmentation mais seulement si les abeilles sont de couleur homogène.

taille d'échantillon / variabilité inter-colonies / variabilité intra-colonies / caractère morphométrique / *Apis mellifera* / *Apis cerana*

Zusammenfassung – Quantitative Analyse zur Bestimmung der intra- und interkolonialen morphometrischen Varianz in Honigbienen, *Apis mellifera* and *Apis cerana*. Mit einer systematischen Analyse wurde die Bedeutung der Probenanzahl in Bezug auf Völker und auf Einzelbienen pro Volk an 6 getrennten und entfernt liegenden Populationen von *Apis mellifera* und *Apis cerana* untersucht. In der Analyse wurden Mittelwerte der Völker, interkoloniale and intrakoloniale Standardabweichungen und Varianzkoeffizienten von 9 morphometrischen Merkmalen der Größe, der Flügeladern und der Pigmentierung benutzt. Das prozentuale Verhältnis der mittleren Varianzkoeffizienten der Völker zur Totalvarianz im Bienenstand

lag für die 6 Standorte zwischen 36 %–116 % (Tab. II). Die Ergebnisse zeigen, dass dieses Verhältnis für die verschiedenen morphometrischen Merkmale und für die Homogenität oder Heterogenität der Bienen innerhalb oder zwischen den Völkern an den verschiedenen Standorten unterschiedlich ist.

Um den Einfluss der Anzahl besammelter Völker auf die Variation der Mittelwerte der 9 Merkmale zu untersuchen wurden in allen möglichen Kombinationen *k* Völker aus einer Gesamtzahl von *n* Völkern an jedem Standort gezogen. Prozentualer Zuwachs oder Abnahme der maximalen oder minimalen Mittelwerte von den geschätzten Populationsmittelwerten der morphometrischen Merkmale zeigte, dass die Variation der Mittelwerte abnimmt, wenn die Anzahl der untersuchten Völker (*k*) zunimmt (Tab. III). Je mehr sich die Bienen in den Farbwerten unterschieden, um so deutlicher war der Effekt der Anzahl untersuchter Völker bei den Färbungsmerkmalen, insbesondere bei kleinen Anzahlen von Völkern (Tab. III, Abb. 1).

Die Zahlen in Tabelle IV demonstrieren in ähnlich eindeutiger Weise einen hohen Grad phänotypischer Stabilität (gemessen als Varianzkoeffizient eines Merkmals) bei den Größenmerkmale oder den Merkmalen der Flügeläderung. Im Allgemeinen waren die Varianzkoeffizienten für diese Merkmale zwischen den Völkern geringer als innerhalb der Völker. Für die Farbmerkmale bestand ein ähnlicher Trend, allerdings war die Anzahl benötigter Einzelbienen drei – bis vierfach höher. Proben von 10 Bienen aus fünf bis 6 Völkern pro Sammelort waren bei den Größen- und Flügeladermerkmalen ausreichend, bei den Farbmerkmalen genügte dies nur wenn die Färbung einheitlich ist.

Kolonieprobenanzahl / Individuenanzahl / Variabilität innerhalb und zwischen Völkern / Morphometrische Merkmale / *Apis mellifera* / *Apis cerana*

REFERENCES

Adams J., Rothman E.D., Kerr W.E., Paulino Z.L. (1977) Estimation of sec alleles and queen matings from diploid male frequencies in a population of *Apis mellifera,* Genetics 86, 583–596.

Alpatov W.W. (1929) Biometrical studies on variation and races of the honey bee *Apis mellifera* L., Q. Rev. Biol. 4, 1–58.

Alpatov W.W., Tjunin F.A. (1925) Beiträge zur Kenntnis der Variabilität der Rüssellänge bei der Honigbiene, Rev. Zool. Russe 5, 79–108.

Cornuet J.M., Garnery L. (1991) Genetic diversity in *Apis mellifera*, in: Smith D.R. (Ed.), Diversity in the Genus *Apis*, Westview Press, Boulder, Colorado, pp. 103–115.

Falconer D.S. (1989) Introduction to Quantitative Genetics, 3rd ed., Longman, Harlow.

Fisher R.A. (1930) The Genetic Theory of Natural Selection, Clarendon Press, Oxford.

Hepburn H.R., Radloff S.E. (1998) Honeybees of Africa, Springer-Verlag, Berlin.

Moritz R.F.A., Klepsch A. (1985) Estimating heritabilities of worker characteristics: a new approach using laying workers of the Cape honeybee (*Apis mellifera capensis*), Apidologie 16, 47–56.

Moritz R.F.A., Southwick E.E. (1992) Bees as Superorganisms, Springer-Verlag, Berlin.

Oldroyd B.P., Moran C. (1983) Heritability of worker characters in the honeybee (*Apis mellifera*), Aust. J. Biol. Sci. 36, 323–332.

Oldroyd B.P., Rinderer T.E., Buco S.M. (1991) Heritability of morphological characters used to distinguish European and Africanized honeybees, Theor. Appl. Genet. 82, 499-504.

Radloff S.E., Hepburn H.R., Neumann P., Moritz R.F.A., Kryger P. (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis*, Apidologie 33, 129–138.

Rinderer T.E., Daly H.V., Sylvester H.A., Collins A.M., Buco S.M., Hellmich R.L., Danka R.G. (1990) Morphometric differences among Africanized and European honey bees and their F_1 hybrids (Hymenoptera: Apidae), Ann. Entomol. Soc. Am. 83, 346–351.

Roberts W.C. (1944) Multiple mating of queen bees proved by progeny and flight tests, Glean. Bee Cult. 72, 281–283.

Ruttner F. (1988) Biogeography and Taxonomy of Honeybees, Springer-Verlag, Berlin.

Statistica for Windows (1995) Graphics, Vol. II, 2nd ed., StatSoft, Inc., Tulsa OK.

Wright S. (1968) Evolution and the Genetics of Populations, Vol. 1, Genetic and Biological Foundations, University of Chicago Press, Chicago.

Wright S. (1969) Evolution and Genetics of Populations, Vol. 2, The Theory of Gene Frequencies, University of Chicago Press, Chicago, Illinois.

Verma L.R. (1992) Honeybees in Mountain Agriculture, Oxford and IBH, New Delhi.