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# A morphometric comparison of samples of female *Varroa jacobsoni* Oud. (*Varroidae; Mesostigmata*) from colonies of European honeybee, *Apis mellifera* L. (*Apidae; Hymenoptera*)

*En morfometrisk sammenligning af hunlige varroamider* (Varroa jacobsoni *Oud.;* Varroidae; Mesostigmata) *fra europæisk honningbi,* Apis mellifera *L.* (Apidae; Hymenoptera)

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## Summary

Samples of female *Varroa jacobsoni* Oud. were compared in order to determine variations in ten morphological characters. A comparison of 26 samples from Europe (incl. Tunis, Turkey), USSR and China showed maximum variations in the characters ranging from 6.2% to 17.6%. Significant evidence of a linear average relationship was found among four pairs of morphocharacters and between one morphocharacter and the precubital index of worker bee wings; such correlations might be usefull when studying mite-bee relations and the genetics of the mite. No correlations was found between morphocharacters and latitude or altitude of location. Genetic drift (especially founder principle and »refounder« principle) is believed to be of great importance in morphological differentiation of populations. Samples from Brazil and Japan and from India deviated from each other and from the rest of the samples in several characters. The morphology of a sample from Japanese *Apis cerana* Fabr. fell within the variation range of the samples from *Apis mellifera* L. Morphotypes of the Varroa mite cannot be defined on the basis of the optained results.


**Key words:** *Varroa jacobsoni,* morphological variation, differentiation.


## Resumé

Prøver af hunlige varroamider sammenlignedes med hensyn til 10 morfologiske karakterer. Mellem 26 prøver fra Europa (incl. Tunis og Tyrkiet), USSR og Kina lå de største variationer i disse karakterer mellem 6,2 og 17,6 pct. Der blev fundet signifikant liniær korrelation mellem fire par morfokarakterer, samt mellem en morfokarakter og arbejderbiers vingebygning (præcubitalindeks); sådanne korrelationer kan muliggøre nye studier af mide-bi-relationer og af midens genetik. Der blev ikke fundet korrelation mellem morfokarakterer og oprindelsesstedets længde- eller breddegrad. *Genetic drift* (især *founder principle* og *»refounder« principle)* anses for at være af stor betydning for midepopulationers morfologiske differentiering. Mideprøver fra Japan og Brasilien og fra Indien afveg i flere karakterer fra hinanden og fra de øvrige prøver. En japansk prøve af mider fra indisk honningbi (*Apis cerana* Fabr.) afveg ikke fra prøverne fra europæisk honningbi (*Apis mellifera* L.). På baggrund af de opnåede resultater kan der ikke defineres forskellige morfotyper af varroamiden.

**Nøgleord:** *Varroa jacobsoni*, morfologisk variation, differentiering.

## Introduction

The parasitic mite *Varroa jacobsoni* Oud. is a major problem almost wherever the European honeybee (*Apis mellifera* L.) is used in beekeeping. In spite of this, very little information on variations in host-parasite relations is published, and although the mite has spread to all continents almost nothing is known about the differentiation of the species.

Morphological variations in adult Varroa females have been reported: The outline of the dorsal shield show variations among Bulgarian samples (6) and between European and Asian samples (7). Besides, variation in position of dorsal setae have been found (12). Mites from *Apis cerana* colonies have been reported to be smaller than those from *Apis mellifera* colonies (4). Comparison of descriptions of the species *Varroa jacobsoni* indicate variations in other characters. A number of irregularities in morphology of individual specimens have been described by *Akimov* and *Yastrebtsov* (1).

This paper describes variations in ten morphocharacters; 30 samples of female mites from different parts of the world are compared. Statistical correlation was analysed in three groups of parameters: 1) between pairs of morphocharacters, 2) between each morphocharacter and each of four types of wing index of worker bees and 3) between each morphocharacter and altitude and latitude of locations.

## Materials and methods

**Samples of female *Varroa jacobsoni* Oud. from colonies of *Apis mellifera* L.**

No. 1 Brazil, Ribeirao Preto; collected 23 Jan. 1986 from africanized bees.

No. 2 Japan, Tokyo; collected 23 Aug. 1985.

No. 3 India, Thalakaveri (Coorg), Karnataka; collected 1985-1986.

No. 4 People's Republic of China, Baoding, Heibei; collected July 1985.

No. 5 Austria, Mistelbach (48.34 N, 16.35 E); collected Oct. 1985 from debris from a single colony.

No. 6 German Democratic Republic, Wurzen (Leipzig) (51.22 N, 12.44 E); collected 26 Aug. 1985 from a single colony.

No. 7 Federal Republic of Germany, Tübingen (48.31 N, 9.02 E); collected 1986 from a single colony.

No. 8 Hungary, Budapest (47.30 N, 19.05 E); collected autumn 1985 from a single colony.

No. 9 Italy, Udine (46.03 N, 13.14 E); collected autumn 1985 from a single colony.

No. 10 Poland, Poznan (52.25 N, 16.55 E); collected summer 1985.

No. 11 Poland, Sandomierz (50.41 N, 21.17 E); collected summer 1985.

No. 12 Poland, Poznan (52.25 N, 16.55 E); collected autumn 1985.

No. 13 Poland, Poznan (52.25 N, 16.55 E); collected autumn 1985.

No. 14 Poland, Pulawy (51.25 N, 21.57 E); collected Sept. 1985.

No. 15 Tunesia, Tunis (36.48 N, 10.11 E).

No. 16 Turkey, Menemen (38.36 N, 27.04 E); collected spring 1987 from colonies in one apiary.

No. 17 Sweden, Tingstäde (Gotland) (57.44 N, 18.36 E); collected 1987 from a single colony.

No. 18 Denmark, Vesterhöjst (55.00 N, 9.00 E); collected 27 May 1987 from brood cells in a single colony.

No. 19 Finland, Monola (61.04 N, 27.40 E); collected June 1985.
No. 20 Federal Republic of Germany, Freiburg (Breisgau) (47.59 N, 7.51 E).
No. 21 Greece, Thessaloniki (40.38 N, 22.56 E); collected summer 1985 from a single colony.
No. 22 Holland, Zutphen (52.08 N, 6.12 E); collected Nov. 1986 from a single colony after treatment with acaricide (Perizin). Colonies were in 1985 moved to this apiary from other parts of Holland.
No. 23 Holland, Zutphen (as sample no 22).
No. 24 Holland, Zutphen (as sample no 22).
No. 25 Austria, Klosterneuburg (48.18 N, 16.20 E); collected Sept. 1985.
No. 26 Austria, Bad-Vöslau (48.34 N, 16.35 E); collected Oct. 1985.
No. 27 Roumania; collected 1979.
No. 28 (Origin unknown); collected 1974.
No. 29 USSR; collected 1985.

**Sample of female *Varroa jacobsoni* Oud. from *Apis cerana* Fabr.**

No. 30 Japan, Komagata-shrine (Nagano Prefecture); collected 27 July 1985.

From each sample ten specimens were mounted (DPX) and examined: 19 preliminary characters were chosen each showing major variations, and means and standard error of the means were computed. Ten morphocharacters with the greatest variability were selected (listed below) and the rest of the specimens (maximum 50; only fully coloured specimens were considered) in the samples were examined in these respects and means and standard error of the means were computed.

**Morphocharacters**

A: Outline of sternale. Width of sternal shield (100 ×) divided by length of sternal shield (400 ×) (Fig. 1: g, f).
B: Index of length of sternale. Length of sternal shield (400 ×) divided by length of frontal margin of sternal shield (400 ×) (Fig. 1: f, e).
C: Position of sternal setae I and II. Distances between sternal setae I and II (left and right side added) (400 ×) divided by distance between sternal setae I (400 ×) (Fig. 1: $h_1$, $h_2$, i).
D: Position of sternal setae II. Distance between sternal setae I and II (left and right side added) (400 ×) divided be distance between adanal setae (400 ×) (Fig. 1: $h_1$, $h_2$, j).
E: Dorso-lateral setae. Number of stout dorso-lateral setae (left and right side added) (Fig. 1: b).
F: Metapodal setae. Number of latero-caudal setae of metapodal shields (left and right side added) (Fig. 1: k). A seta is not counted if the with of the basal halo is smaller than the distance from the halo to the margin of the shield.
G: Spacing of metapodal setae. Character F divided by measures of metapodal shields (left and right side added) (100 ×) (Fig. 1: l).
H: Position of dorso-lateral setae. Distance between foremost dorso-lateral setae in left and right side (50 ×) divided by distance between adanal setae (400 ×) (Fig. 1: n, J).
I: Spacing of dorso-lateral setae. Distance between foremost and hindmost dorso-lateral setae (left and right side added) (50 ×) divided by character E (Fig. 1: $m_1$, $m_2$).
J: Position of gnathosomal setae. Distance between middle and proximal gnathosomal setae (left and right side added) (400 ×) divided by distance between middle and distal gnathosomal seta (both sides added) (400 ×) (Fig. 1: $p_1$, $p_2$, $o_1$, $o_2$).

These morphocharacters are based on numbers of setae on shields, on distances between setae on shields or on solid body parts and on measures of shields; influences from different ways of drying and from different preservation fluids applied to the samples are thus minimized.

Possible correlations among the morphocharacter means of 22 samples (no. 5–17, 19–27) were analysed by means of t-tests each involving 2 characters; 40 tests were carried out (if both characters were computed on the basis of the same count/measure the combination of characters was not analysed).

For the tests, t-values were computed as

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}},$$

(r = correlation coefficient) (2); n – 2 degrees of freedom and 5% level of significance were used. Critical value of t is 2.262.

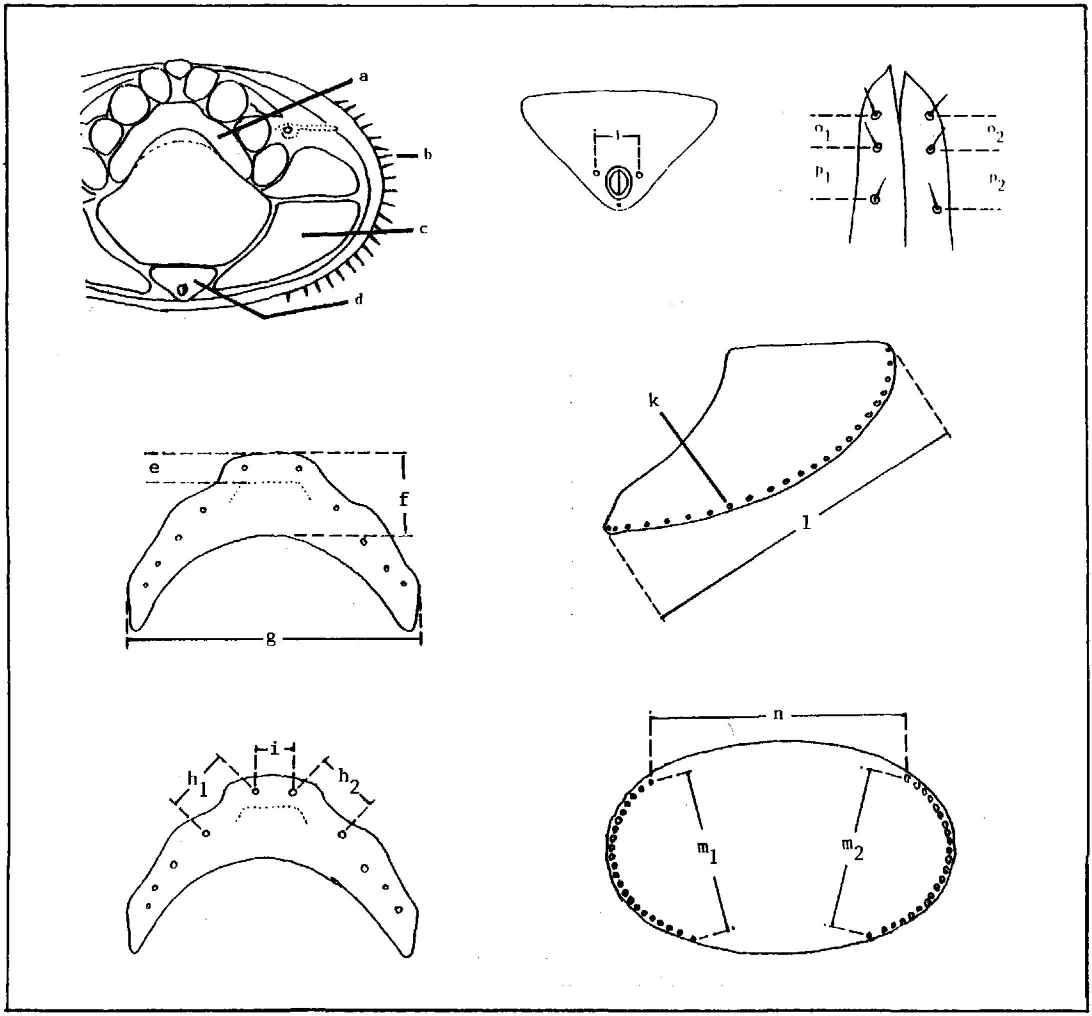



Fig. 1. Morphology of female *Varroa jacobsoni* Oud. e-i: characters of sternal shield (a); j: character of anal shield (d); k-l: characters of metapodal shield (c); m-n: characters of stout dorso-lateral setae (b); o-p: characters of gnathosome.

*Morfologi hos* Varroa jacobsoni *Oud. (hun). e-i: sternale (a); j: anale (d); k-l; metapodale (c); m-n: dorso-laterale setae (b); o-p: gnathosome.*

Each of 11 samples were supplemented with a number of worker bees (Table 1) from the infested colonies. On the basis of measurements of the right forewing of these bees, four types of wing index were computed: cubital index, precubital index, radial index and index of cubital cells (for definitions of these indexes see *Chauvin* (3)). Possible correlation between each index mean and each morphocharacter mean was analysed by means of t-tests (as above); 40 tests were carried out.

Data from 20 samples (no. 5–16, 19–26) were used in analyses of possible correlations between each morphocharacter mean and latitude and between each morphocharacter mean and altitude of locations (t-tests as above); 20 tests were carried out.

**Table 1.** Number of worker bees and their precubital index.
*Antal arbejderbier og deres præcubitalindeks.*

| Sample<br>*Prøve* | Number of worker bees<br>*Antal bier* | Precubital index ($\bar{x} \pm$ S.E.M.)<br>*Præcubitalindeks* |
|---|---|---|
| 5. Austria | 40 | 3.651 ± 0.123 |
| 6. GDR | 35 | 3.182 ± 0.080 |
| 7. FRG | 47 | 3.335 ± 0.063 |
| 8. Hungary | 24 | 3.434 ± 0.134 |
| 9. Italy | 50 | 3.149 ± 0.051 |
| 10. Poland | 24 | 3.467 ± 0.119 |
| 11. Poland | 20 | 4.672 ± 0.255 |
| 12. Poland | 26 | 4.089 ± 0.207 |
| 13. Poland | 23 | 3.385 ± 0.119 |
| 14. Poland | 21 | 3.509 ± 0.116 |
| 15. Tunesia | 50 | 2.652 ± 0.044 |

# Results

The means and standard error of the means of the morphocharacters A–J are shown in Fig. 2A–J.

The samples from Brazil (no. 1) and Japan (no. 2) diverge clearly from the main part of samples (no. 4–29) in morphocharacters A, B, F, G and J; the Indian sample (no. 3) diverge from the main part of samples in character A, B, E, F, G and J. The main part of the samples originate from Europe (no. 5–14, 17–27), Tunesia (no. 15), Turkey (no. 16), USSR (no. 29) and from China (no. 4). In this group of samples the variations in morphocharacters ranges from 6.2 p.c. (character A) to 17.6 p.c. (character C). In a number of characters, samples originating from limited geographical areas show variations among themselves equalling or exceeding the maximum variations of the rest of the European samples (no. 5 and 26 in character B; no. 10, 12 and 13 in character C, D and G).

Statistical analyses of the correlation among morphocharacters of samples from Europe (incl. Tunesia and Turkey) show significant evidence of linear average relationships between four pairs of morphocharacters (Fig. 3).

Analyses of correlations among morphocharacters and types of wing index of worker bees, show evidence of a linear average correlation between means of precubital index (Table 1) and means of morphocharacter H ($t = 2.474$; $P > 0.05$). No correlation was found among morphocharacters and altitude or latitude of locations.

The sample (no. 30) from a colony of Japanese honeybee *(Apis cerana)* is not in any of the morphocharacters clearly separated from samples of mites from European honeybee *(Apis mellifera)*.

In all samples the stout dorso-lateral setae were strait or slightly curved – never sickle shaped. A postanal seta was present in all samples.

# Discussion

The uneven level of information on method, date etc. of collection of the mites, attaches some uncertainty to the comparability of the morphological variations, and put certain limits to what statistical analyses can reasonably be applyed to the variations.

Morphological variations among samples of Varroa females may be caused by a) different influences from the environment and by b) differences in genetics of the populations.

Different environmental influences may be caused by locality – which probably will be blurred by migratory beekeeping – and by physical conditions in the nest of the bees. The physical conditions in the nest are highly controlled by the bees themselves and may change in case of requeening or queen supersedure and if abnormal conditions occur (starvation, drought, disease).

The genetics of a Varroa population is subject to changes caused by selective pressures influencing and influenced by the host-parasite relations. It has been found that the genetics of the bee colony differ in a character which will influence the mite (13), so in case of requeening or queen supersedure the selective pressures on the Varroa population can be expected to change.

Fig. 2. Means and standard error of the means of morphocharacters A-J. Samples no. 1-30 are lined from left to right; samples no. 5, 15 and 25 have their number above markings. Figures below the markings give the number of examined mite specimens. →

*Mideprøvernes morfokarakterer A-J ($\bar{x}$ ± S.E.M.). Prøverne (nr. 1-30) er anført i nummerfølge fra venstre; for prøve nr. 5, 15 og 25 er prøvens nummer angivet over værdimarkeringen. Tallene under værdimarkeringerne angiver antal undersøgte mider.* →

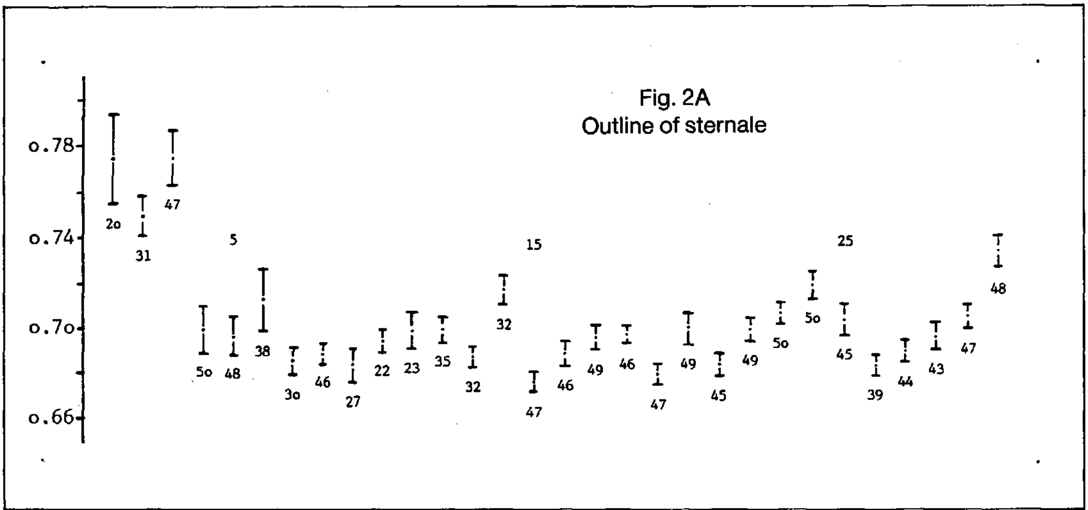



Fig. 2A
Outline of sternale

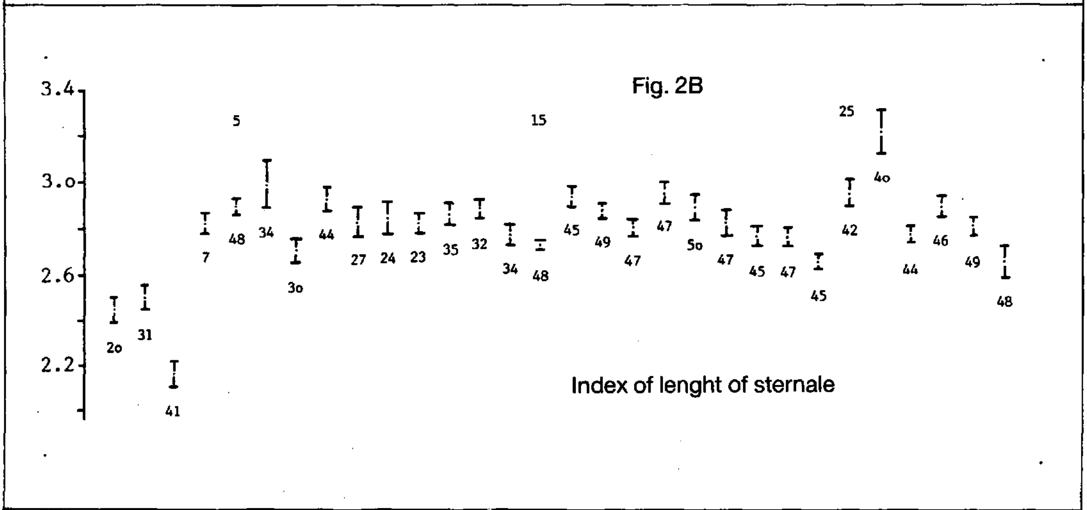



Fig. 2B
Index of lenght of sternale

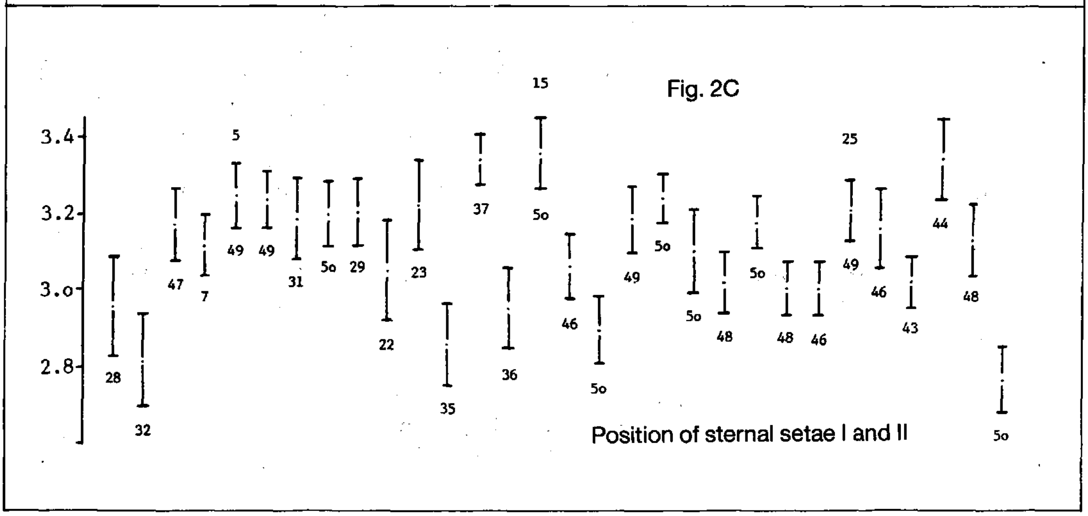



Fig. 2C
Position of sternal setae I and II

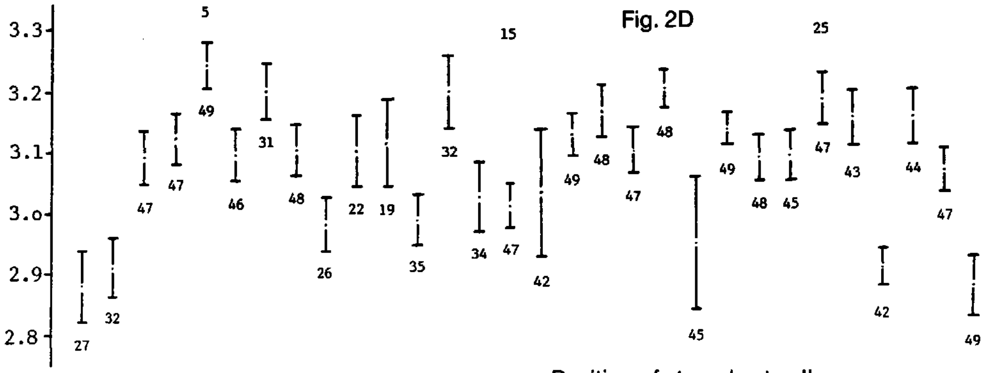



Fig. 2D

Position of sternal setae II

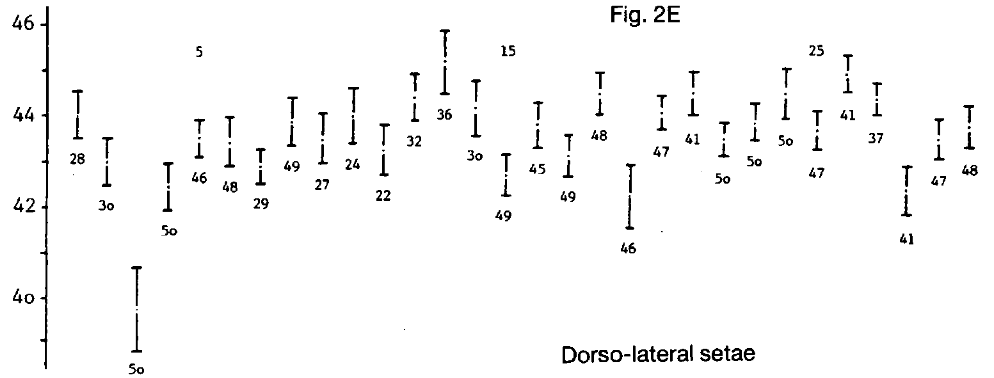



Fig. 2E

Dorso-lateral setae

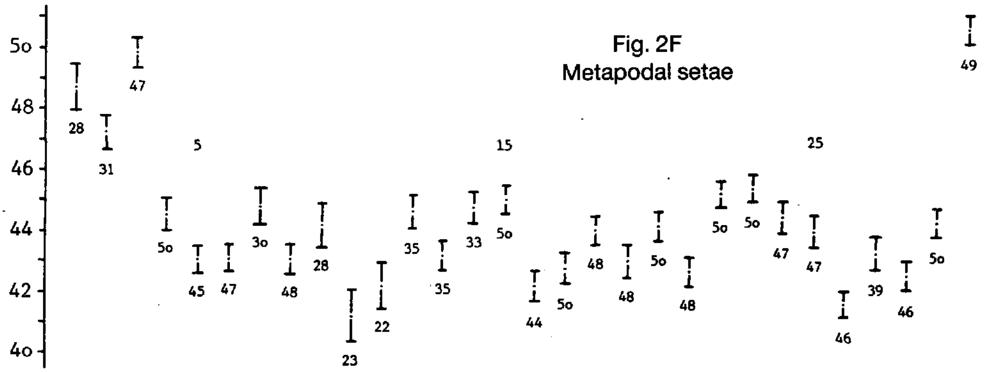



Fig. 2F
Metapodal setae

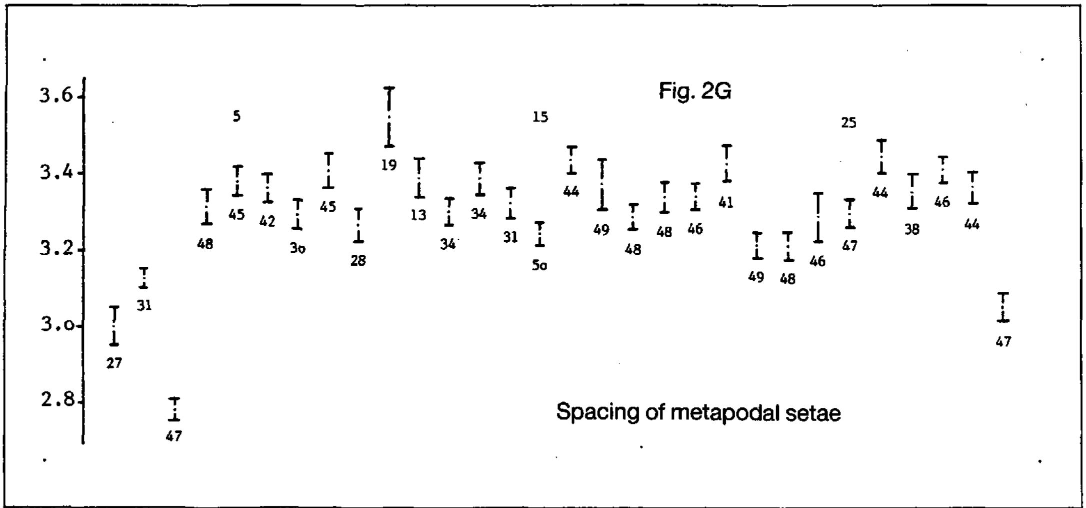



Fig. 2G

Spacing of metapodal setae

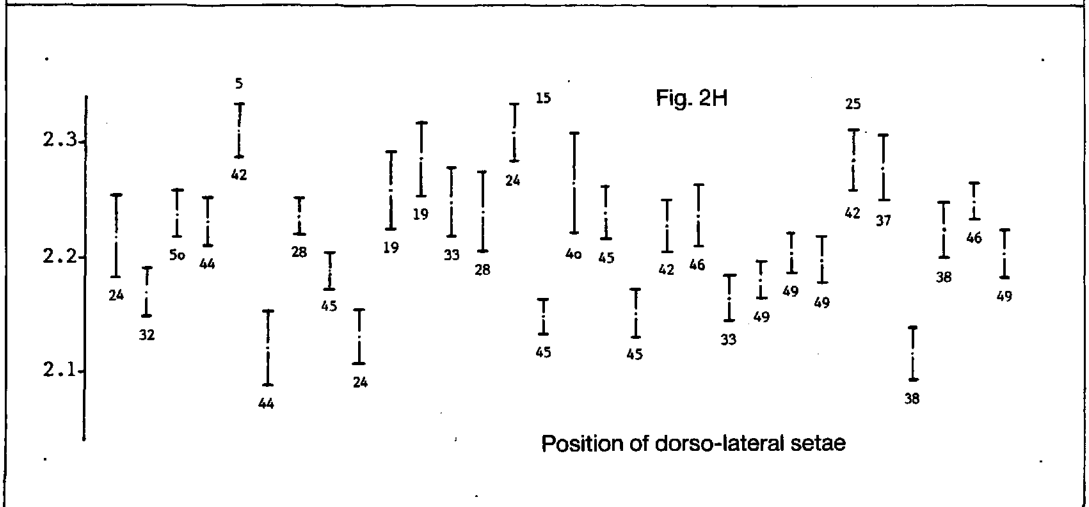



Fig. 2H

Position of dorso-lateral setae

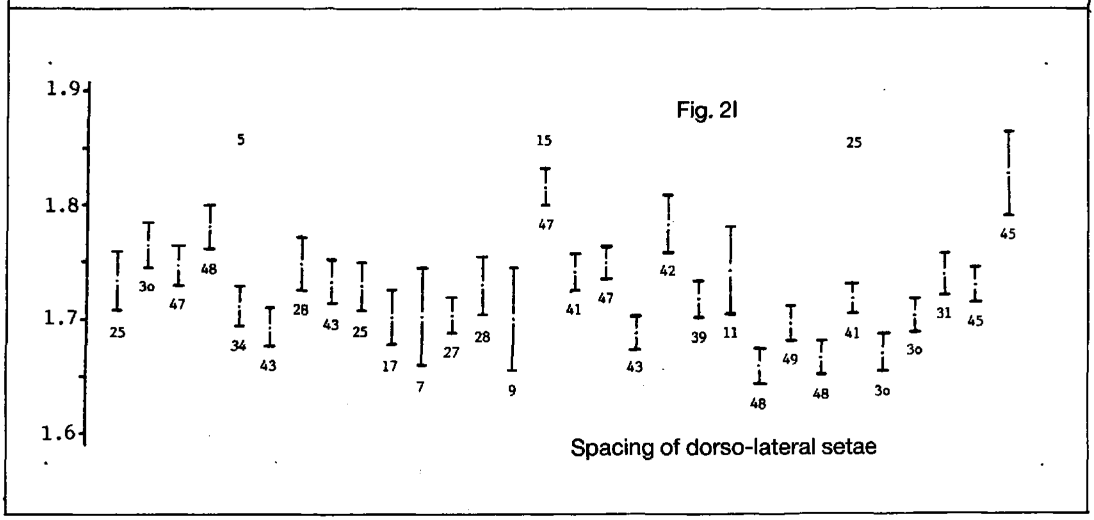
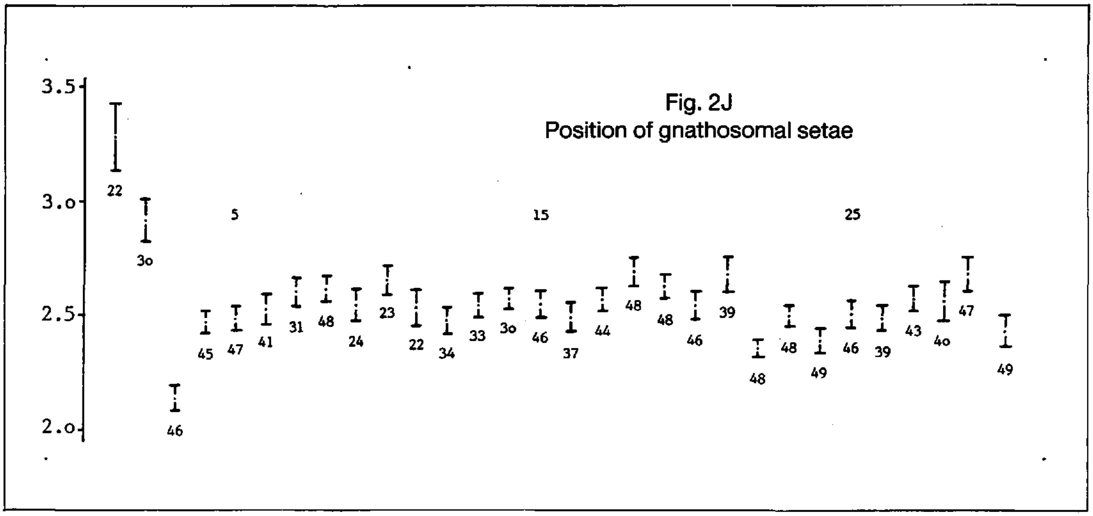



Fig. 2I

Spacing of dorso-lateral setae




The genetics of a Varroa population can also be changed by non-selective mechanisms. The characteristics of a founding population will greatly influence the proporties of the resulting population; treatment of infested colonies will often leave a small number of mites which can be regarded as a »refounding« population. The size of the actual reproducing Varroa units is very small: In most cases mating occur between offspring from a single female mite (offspring consisting of 1–5 females and 1 male) and only when more than one female invade the same brood cell mating between members of different offspring »families« is possible. Migration or transportation of mites from one colony to another may lead to coexistence of subpopulations within the bee colony; gene flow between such subpopulations will normally be small. However, queen rearing is followed by a marked increase in the number of brood cells containing more than one invading female (8) and natural queen supersedure will probably increase the gene flow between subpopulations to a higher degree than artificial queen replacement normally does. Modern bee colony management including treatments intending small number of mites and artificial (rather than natural) queen replacement is expected to decrease the mixing of subpopulations and to increase the differentiation of mite populations. The great morphological variations among samples from limited geographical areas support this opinion.

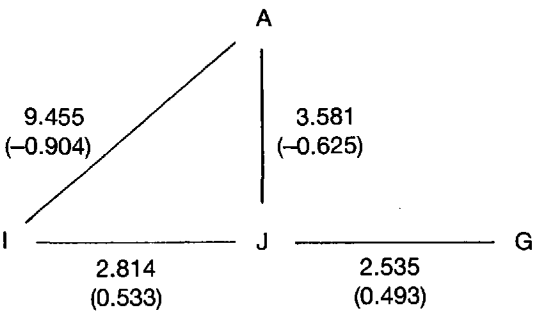



Fig. 3. The relationships among the morphocharacters A, I, J and G; each line symbolize a significant linear average correlation. The figures give values of t; the figures in parentheses give values of correlation coefficients (r). A positive r-value shows that the values of the characters increases together and a negative r-value shows that the value of one character decreases as the other increases.

*Sammenhæng mellem fire morfokarakterer A, I, J og G. Hver linie symboliserer en significant korrelation. Tal angiver t-værdier og tal i parentes angiver korrelationskoefficienter (r). En positiv r-værdi viser proportionalitet og negativ r-værdi viser omvendt proportionalitet mellem morfokarakterernes talværdier.*

The genetic system of the Varroa mite and the small actual reproducing units make it probable that non-selective mechanisms (genetic drift: founder principle and proporties of »refounding« populations) are of great importance in the process of differentiation. Non-selective mechanisms' great influence is indicated by the apparently random fluctuations in most of the morphocharacters: 36 of 40 pairs of morphocharacters showed no correlation with each other. This is, however, only indicated, since most of the morphocharacters may be correlated to characters that are not considered in this study.

The find of four significant correlations between pairs of morphocharacters is promising; these correlations might – after detailed studies – prove to be usable in genetic works. The significant correlation between a morphocharacter of the mite and a morphological character of the bees is rather surprising and needs further elucidation; the morphology (cubital index) of worker bee wings is known to depend on the conditions during the development (14). Correlations between morphology of mites and bees might be used as markers in studies of the host-parasite relations.

The deviating morphological characteristics of the samples from Japan, Brazil and India suggest that three morphotypes – an European-Asian, an Indian and a Japanese-Brazilian – may be distinguished, but as morphovariations among samples of each morphotype must be expected to be as great as among the samples from Europe, no morphotypes can be defined on the basis of the limited material used in this study.

The Varroa mite was introduced into West Germany with bees originating from either South-/East Asia or from the Balkans (11); the present results do not disfavour a Balkan origin. It is not possible to deduce any information on the routes of spread within Europe from the obtained results. The general accepted view that the mite has been introduced into Tunesia and Turkey from the Balkans and into South America from Japan is not contradicted by the present results.

No case of strongly curved dorso-lateral setae was found, and finds of such setae (5, 9, 10) are regarded as artefacts caused by treatment with softening agents. The information given by *Liu* (9) that Brazilian mites lack the postanal seta was not confirmed.

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