

CORRESPONDENCE ANALYSIS OF LARVAL CHAETOTAXY IN THE « *ANOPHELES MACULIPENNIS* COMPLEX » (DIPTERA, CULICIDAE).

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SUMMARY

Relationship between several species such as *A. atroparvus*, *A. subalpinus*, *A. maculipennis*, *A. messeae* from the « *Anopheles maculipennis* complex » were studied by means of Correspondence Analysis (CA). The analysis was performed on chaetotaxic data collected in Lyon area (France), and on data from literature on French and Italian populations.

CA shows clusters among the whole frequency distributions set,

on the basis of sample means and sample distribution shapes. CA appears to be more sensitive to both criteria, compared to usual statistical tests. The results show the acuity of the method.

Our study confirms that larval chaetotaxy is an adequate taxonomic character. Its use can be extended to the detection of mixed populations of several species and to the identification of new or litigious larvae strains.

RÉSUMÉ : Chétotaxie larvaire du « complexe *Anopheles maculipennis* » (Diptera, Culicidae). Étude par Analyse Factorielle des Correspondances.

La proximité des espèces *A. atroparvus*, *A. subalpinus*, *A. maculipennis*, *A. messeae* du « complexe *Anopheles maculipennis* » est étudiée par Analyse Factorielle des Correspondances (AFC) pratiquée sur des données chétotaxiques obtenues sur des populations larvaires de la région de Lyon (France) mais aussi sur d'autres populations françaises et italiennes, issues de données bibliographiques.

L'AFC opère un regroupement des histogrammes de répartition

des soies antépalmées selon leur moyenne et leur forme avec une sensibilité supérieure à celle obtenue par examen des moyennes et des variances des échantillons.

Nos résultats confirment la valeur taxonomique de la chétotaxie larvaire et en élargissent les possibilités d'utilisation (détection de mélanges d'espèces, identification de souches nouvelles ou incertaines...).

INTRODUCTION

« *Anopheles maculipennis* » is a biosystematic complex of « sibling species » (Mayr, 1942).

Palaearctic members can be identified by morphological characters of their eggs and larvae, by cytogenetic criteria (unfortunately not very discriminating) or at the present time, by isoenzymes.

The study of eggs, as a first step towards identification is often subjective (Guy *et al.*, 1977). Larval chaetotaxy permits a more reliable identification but is limited: the mean total number of antepalpal hair branches of IVth and Vth abdominal segments, when estimated from large samples of fourth instar larvae, varies (Bates, 1939) among

species. Based on this character, the observed sample frequency distributions pretty often show overlappings of a large extent, and their ranges, means and variances are of closed values, particularly in *A. maculipennis* and *A. messeae*. This may cause ambiguous identification. Guy *et al.* (1976), Suzzoni-Blatger *et al.* (1982) used the probability of belonging to one species in given ranges of variation as a discriminating criterion. This last author recently considered this criterion as mediocre because submitted to large intraspecific geographical variations. Generally speaking, some strains, *i. e.* the larvae originating from one female, may remain unidentified after morphological study of eggs and larvae (about 5 % of the strains according to Suzzoni-Blatger *et al.*, 1990). To analyse the many chaetotaxic data obtained from « *Anopheles maculipennis* complex » of the Lyon area (Pichot *et al.*, 1981, Deruaz, 1988), the Correspondence Analysis or CA (Benzecri, 1973, Greenacre, 1984) was preferred to the inferential methods usually used (χ^2 -square tests, analysis of variance). CA permits the simultaneous analysis and representation of several frequency distributions of a same kind, using the multidimensional

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mensional information lying among them. Distributions are plotted as points on optimal planes, with the distance between points or clusters of points giving account of the « biological distance » between the tested data.

Testing the interaction between CA and chaetotaxic distributions is of interest: if the species can be properly separated by CA, on the basis of chaetotaxy, then the number of antepalpmate hair ramifications is confirmed as an operative criterion. Revertedly, the accounted adequacy of CA to discrimination among the « *Anopheles maculipennis* complex » leads to fruitful applications: a previous stock of CA analysed data can be used as a reference for further strain controls or identifications.

MATERIAL AND METHODS

AREA OF MOSQUITOES COLLECTION

Insects originate from habitats (resting places) referred in Roman (1963, 1971a et 1971b) and Pichot, 1978. Females were collected in six places of the Dombes (French department 01), the plaine du Forez (French department 42) and the Bas Dauphiné (French department 38), inside four types of feeding-sites: cow-sheds, sheep folds, pigsties, kennels (Table II and Fig. 1 for geographical localization).

IDENTIFICATION CRITERIA

In the laboratory, each egg-laying was totally examined and classified according to the type of chorionic ornamentation, then was reared separately. The total number of antepalpmate hair branches was recorded on the fourth instar larvae:

— For each strain, the mean number was estimated, then compared to reference values (Table I).

— For each population (*i. e.* larvae from females collected in one place and giving the similar lay) the distribution of data was represented by an histogram.

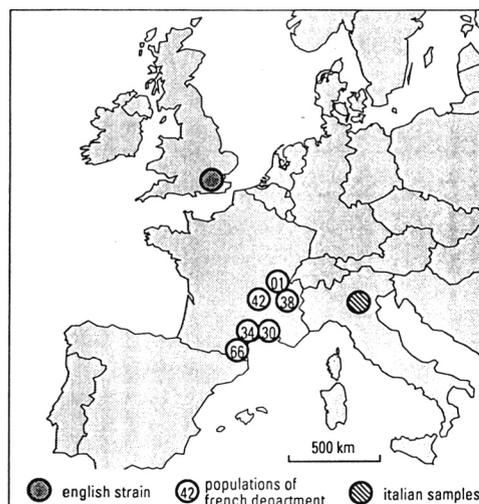


FIG. 1. — Localization of mosquitoes sampling areas in England, Italy and France (french departments: 01 = Ain, 30 = Gard, 34 = Hérault, 38 = Isère, 42 = Loire, 66 = Pyrénées-Orientales).

It is notable that confusion between *A. subalpinus* and *A. messeae* species may linger, even after study of these two morphological criteria, as chorionic ornamentations are quite similar and ranges of antepalpmate hair branches number too (Table I). According to the bibliographic synthesis performed by Suzzoni-Blatger *et al.*, 1982 about chaetotaxic results, *A. subalpinus* may lead to samples with a mean number of antepalpmate hair branches higher than 20, while the other species never do. So we chose to label a given strain as *A. subalpinus* only if the eggs presented strongly marked bars and mottles and led to a larvae group with at least 20 as a mean value.

Statistical analysis

Data were analysed using « MACMUL » and « GRAPHMU » computer programs (Thioulouse, 1989).

TABLE I. — Previously published larval chaetotaxy of the « *Anopheles maculipennis* complex »: reference values.

antepalpmate hair branches species N°	BATES (1939)			SUZZONI-BLATGER et al. (1982)			GUY et al. (1976)		SUZZONI-BLATGER et al. (1982)	
	mean	standard deviation	maximum range	mean	standard deviation	maximum range	range	N° of mosquitoes (%)	range	N° of mosquitoes (%)
<i>A. atroparvus</i>	10,80	1,62	7-16	11,56	2,04	7-22	9 - 14	89%	9 - 14	84%
<i>A. maculipennis</i>	13,05	1,35	10-16	13,74	2,14	7-23	11 - 16	85%	11 - 16	85%
<i>A. messeae</i>	14,82	2,40	11-21	15,88 18,10	2,10 2,42	11-27	11 - 17	85%	13 - 18	63%
<i>A. subalpinus</i>	24,49	3,30	16-32	19,29	—	12-28	20 - 29	79%	20 - 28	58%

TABLE II. — Contingency table submitted to CA: distributions of the number of antepalate hair branches. 19 × localizations in line; 13 size classes in column.

Species	Dpt	Localization	N°	Total N° of antepalate hair branches (VI larvae, IVth and Vth abd. seg.) :													Total sampled
				7	10	12	13	14	15	16	17	18	19	21	25	31	
				9	11	classes n° :										20	
				1	2	3	4	5	6	7	8	9	10	11	12	13	
A. messeae	LOIRE	Mornand (cow-shed) (pigsty)	1	1	4	18	13	30	23	41	35	24	46	23	2	1	261
			2	0	10	19	20	21	23	30	22	21	29	18	0	0	213
	AIN	Servas (cow-shed)	3	0	0	9	15	14	16	22	16	21	10	9	1	0	133
		Birieux (kennel)	4	7	24	50	35	49	59	58	51	38	44	27	3	0	445
A. maculipennis	ISERE	Meyrieux-les-Etgs St Jean-de-Bournay (cow-shed, sheep fold)	5	0	1	5	7	22	12	27	19	20	39	25	0	0	177
		LOIRE	Mornand (cow-shed) (pigsty)	6	4	3	13	15	6	3	3	4	2	5	2	0	0
7	0		4	3	3	6	6	3	4	2	0	0	0	0	0	31	
A. subalpinus	ISERE	Meyrieux-les-Etgs St Jean-de-Bournay (cow-shed, sheep fold)	8	0	13	45	42	45	41	13	13	7	5	0	0	0	224
		LOIRE	Mornand (cow-shed) (pigsty)	9	0	0	0	0	0	0	4	3	5	13	17	7	3
10	0		0	0	0	0	0	0	0	1	1	7	3	1	0	13	
A. subalpinus	AIN	Servas (cow-shed)	11	0	0	0	0	1	0	0	5	2	13	20	13	3	57
		ISERE	Meyrieux-les-Etgs St Jean-de-Bournay (cow-shed, sheep fold)	12	0	0	0	0	0	0	0	0	2	3	5	4	1
A. atroparvus	66		Argelès/mer	13	23	39	80	21	13	5	2	1	2	1	1	0	0
		Astouin	14	25	33	47	13	7	4	0	0	0	0	0	0	0	0
A. subalpinus	34	Bas Languedoc	15	0	0	0	0	1	2	6	4	10	28	90	113	27	281
		30	Astouin	16	0	0	0	0	2	2	4	4	25	70	177	96	14
	Italy		17	1	3	7	4	7	8	8	8	9	17	21	5	3	101
A. messeae		Italy	18	3	12	22	15	11	11	10	6	3	3	2	0	0	98
A. maculipennis		Italy	19	2	10	26	17	13	9	9	6	1	3	0	0	0	96

1-3 : bibliographical data.

Dpt : French department
(name or N° : 30=Gard; 34=Hérault; 66=Pyrrénées orientales).

STANDARDISATION OF THE METHOD ON THE BASIS OF REFERENCE POPULATIONS

SAMPLES (Tables II and III)

From 1681 out of 2412 fourth instar observed larvae, we studied 12 distributions of antepalate hair number, obtained in different places and relating to *A. maculipennis*, *A. messeae*, and *A. subalpinus* (no. 1 to no. 12).

Seven distributions of chaetotaxic data, relating to 1287 larvae, were found in the literature and added to our own stock of data:

— two samples from the « Midi méditerranéen », stu-

TABLE III. — Larval chaetotaxy: correspondence between the classes no. and the hair branches.

Classé N° :	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of antepalate hair branches :	7-9	10-11	12	13	14	15	16	17	18	19-20	21-24	25-30	31-37

died by Rioux (1958) related to *A. atroparvus* (no. 13) and *A. subalpinus* (no. 15);

— two samples from Astouin (French department 30, see Fig. 2), of *A. atroparvus* (no. 14) and *A. subalpinus* (no. 16) studied by Salières *et al.* (1978);

— three Italian sample from Frizzi in Rioux (1958) of *A. subalpinus* (no. 17), *A. messeae* (no. 18) and *A. maculipennis* (no. 19).

. CHAETOTAXIC DISTRIBUTIONS

The numbers of antepalpmate hair branches ranged from 7 to 37 in the samples. 13 classes were built (Table III).

Table II shows the data submitted to CA: the columns are the 13 size classes and the rows are the species according to their geographical origin.

STUDY OF NEW POPULATIONS

. PROJECTION OF SUPPLEMENTARY INDIVIDUALS

The distribution of each population was analysed by CA as one individual. Additionnal distributions can be graphically represented onto the main reference planes previously obtained from the reference distributions. The position of the additionnal distributions relatively to the reference ones can then be used to identify the species of litigious populations.

. STUDIED ADDITIONNAL DISTRIBUTIONS

The distributions of the following populations were compared to the 19 reference distributions:

— 8 populations from Lyon area, difficult to identify by means of chaetotaxy:

. populations suspected to be *A. atroparvus* (a1, a2, a3, a4) but showing contradictory results after the study of eggs and larval hairs,

. populations suspected to be *A. subalpinus* (s1, s2, s3, s4);

— two populations described in Rioux (1958):

. a strain *A. atroparvus* from South of France (R2);

. a mixture (R1) of two mediterranean populations of *A. atroparvus* (1.8 %), *A. subalpinus* (97.8 %) and their hybrids (0.4 %);

— an English strain of *A. atroparvus* (aa), reared in our laboratory (Table IV).

RESULTS

STUDY OF THE 19 REFERENCE POPULATIONS

The F1F2 plane provides the less distorted image of the columns and shows that:

TABLE IV. — Antepalpmate hair branches distributions of new « Anopheles maculipennis complex » populations considered as supplementary individuals in CA.

Species	Localizations	Name	size classes													Dpt	Total sampled
			1	2	3	4	5	6	7	8	9	10	11	12	13		
<i>A. atroparvus</i> ?	Mornand: cow-shed	a1	1	2	7	4	6	4	2	4	1	4	4	1	0	LOIRE	40
	Mornand: pigsty	a2	0	0	0	0	0	0	0	2	1	2	1	0	0		
	Birieux : kennel	a3	6	9	16	13	25	24	22	15	13	25	12	1	0	ISERE	30
	Meyrieux-les-étangs : cow-shed, sheep fold	a4	0	1	5	10	5	3	2	1	3	0	0	0	0		
<i>A. subalpinus</i> ?	Mornand: cow-shed	s1	1	3	14	24	17	27	23	20	22	32	12	1	1	LOIRE	197
	Mornand: pigsty	s2	0	0	0	0	0	0	0	36	33	31	23	1	0		
	Servas : cow-shed	s3	0	0	0	1	1	4	0	0	1	5	7	1	0	GARD	224
	Birieux : kennel	s4	0	1	1	7	2	5	4	1	5	1	2	0	0		
<i>A. subalpinus</i> - <i>A. atroparvus</i> (mixed population of the species+hybrids)		R1	0	1	2	1	1	2	3	3	7	19	56	99	20	GARD	196
<i>A. atroparvus</i> (strain from the "Midi méditerranéen")		R2	75	55	53	8	3	2	0	0	0	0	0	0	0	GARD	50
<i>A. atroparvus</i> (English strain)		aa	34	12	4	0	0	0	0	0	0	0	0	0	0		

Dpt : French departments (see Figure 1 for localization).

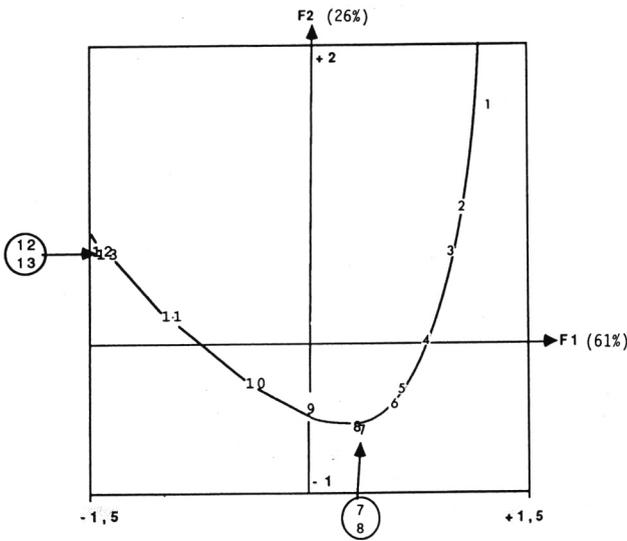


FIG. 2. — Larval chaetotaxy studied by CA: projection of the 13 size classes by the F1F2 plane; « Guttman » effect and ordination on classes by F1 axis. Central and extreme classes are opposed by F2 axis.

— projected points turn to be ranked from 1 to 13 on the F1 axis. This has to be connected to the relative order of sample means; therefore, this axis is considered as a « size » axis;

— F2 axis shows a differentiation between central classes (5 to 9) and extreme ones (1-2-3) and (12-13). This indicates that the F2 axis relates to the shape of the histograms.

In addition, plotted points lie along a parabolic curve. This is known as the « Guttman » effect usually observed with overlapping histograms of closely related modes (Persat *et al.*, 1989, Wartenberg *et al.*, 1987).

On Figures 3 and 4, which represent the 19 populations (the « lines-distributions ») respectively plotted on the F1F2 and F2F3 planes, species are clearly separated into four groups:

— on the F1 axis, the « size » one, the S group (*A. subalpinus*) with a large mean is opposed to the A group (*A. atroparvus*) with a low mean;

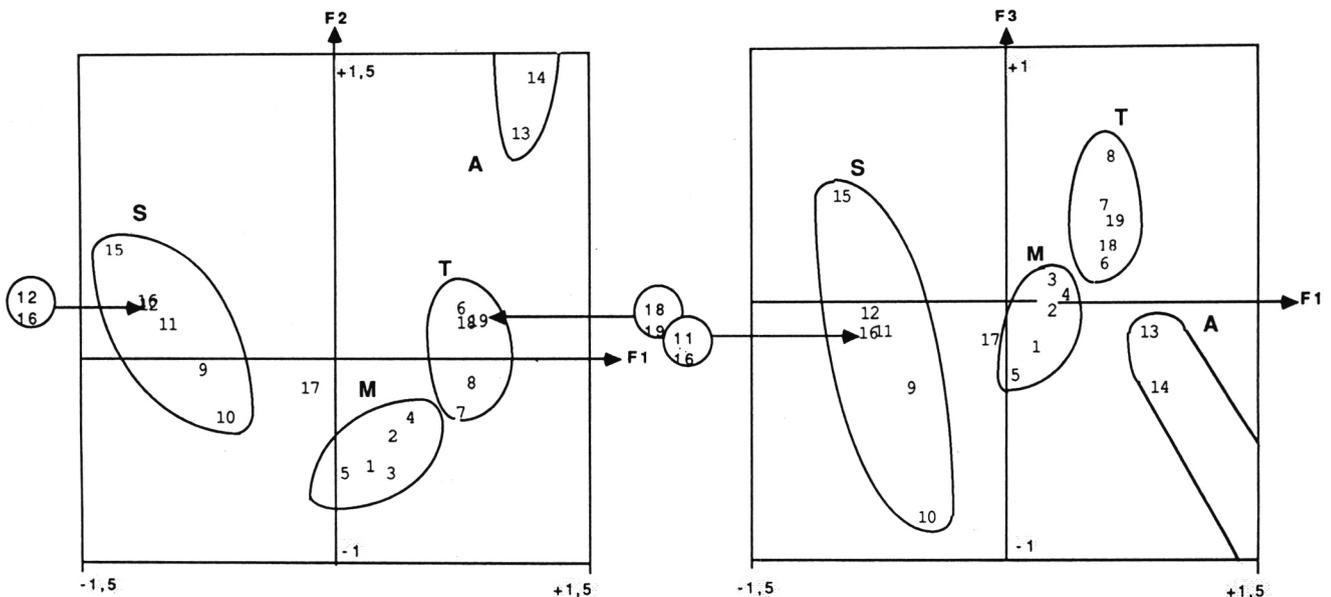
— the F2 axis, the « shape » one, permits to oppose histograms with modes in central classes to those with a single mode out of the central classes: the M (*A. messeae*) and T (*A. maculipennis*) groups show rather symmetrical distributions with unpronounced maxima in classes 5 to 9, and are opposed to the S (*A. subalpinus*) and the A (*A. atroparvus*) groups, showing a dissymmetrical distribution with an accentuated mode in extreme classes;

— the F3 axis permits to isolate the T group (*A. maculipennis*) from others: this axis separates the histograms having their modes in the 4-5-6 or 12-13 classes from other histograms.

STUDY OF NEW POPULATIONS

Study of the F1F2 plane (Fig. 5) and of the following ones, F1F3, F1F4 (not represented here) shows that:

— Lyon populations that were supposed to be *A. atroparvus* would be actually *A. messeae* for a3, *A. subalpinus* for a2 and *A. maculipennis* for a4;



FIGS 3 and 4. — Larval chaetotaxy studied by CA: projection of the 19 populations in the F1F2 and F2F3 planes. F1 arranges the samples in order of their means, F2 and F3 specify their form. Four groups become individualized: S: *A. subalpinus*, M: *A. messeae*, T: *A. maculipennis*, A: *A. atroparvus*.

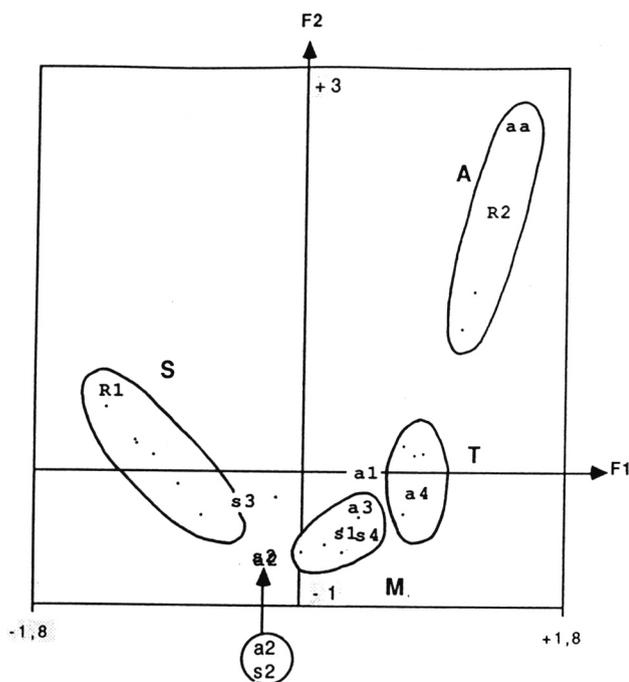


FIG. 5. — Larval chaetotaxy studied by CA. F1F2 plane: projection of 11 populations as supplementary individuals in the analysis. The following planes (F1F3, F1F4... not represented here) have been taken into account to choose the suitable labels.

— populations supposed to be *A. subalpinus* would rather be *A. messeae* (s1 and s4) or even mixed populations of *A. messeae* and *A. maculipennis* species (s4 according to the F3 and F4 axes);

— it is confirmed that s2 and s3 belong to the *A. subalpinus* group;

— the « Midi méditerranéen » *A. atroparvus* (R2) and English (aa) strain which turn to be eccentric on the plot, but closed to the *A. atroparvus* group area (high homogeneity and low modal value) seem to belong to this group;

— the R1 mixed population of *A. atroparvus* and *A. subalpinus* species is undoubtedly associated to the *A. subalpinus* group. Its position on the plot is slightly oriented towards the *A. atroparvus* group whereas other populations belonging to the *A. subalpinus* group are plotted onto the parabolic curve, indicator of the « Guttman » effect.

DISCUSSION

STUDY OF THE 19 REFERENCE POPULATIONS

Projecting the data distributions onto successive CA planes reveals a clear separation between the four species, without any notable variation due to the biotope. Reference samples from Lyon area are closely related to the samples from the literature.

However, two main differences can be pointed out:

1) The projection of the *A. subalpinus* Italian population no. 17 lies out of the S group zone, and towards the M and T groups. We consider that this larval population was not of *A. subalpinus* species only, but a mixed population of *A. subalpinus*, *A. messeae* and/or *A. maculipennis* individuals. This population cannot be made out of hybrids from *A. maculipennis* × *A. subalpinus* or *A. messeae* × *A. subalpinus* crossings since such crossings lead to a high larval mortality (Kitzmilller *et al.*, 1967).

Another hypothesis would be the existence of a particularly large variation of geographical origin for this population.

2) The *A. messeae* Italian population no. 18, is plotted within the T group zone. This population thus should be of *A. maculipennis* species.

CA reveals histogram clusters mainly according to their means and shapes, with a better sensitivity than usual analyses (either comparing means and variances, or performing « variance analysis »). Of course, it separates *A. subalpinus* from *A. atroparvus* since their unimodal distributions differ by their means and the reversed dissymmetry. But comparing histogram shapes becomes especially interesting to separate *A. messeae* from *A. maculipennis*. Both species distributions show similar multiple central modes (classes 3-7 corresponding to 12-17 hair branches) which are slightly accentuated and, as a consequence, highly sensitive to data collecting imponderables. For these two species, CA shows two facts:

— not accentuated modes appear in classes 5-6-7 (14-15-16 hair branches) for the *A. messeae* species while they stand in classes 3-4-5-6 (12-13-14-15 hair branches) for *A. maculipennis*;

— the mode appearing in the classes 9-10 (18 and 19 to 20 hair branches) of *A. messeae* species is quite reduced for *A. maculipennis*.

STUDY OF NEW POPULATIONS

Projecting new sample distributions as supplementary individuals provides informations on the composition of these samples, from breeding or field collecting. The technique permits the reliable identification of species and the possible detection of mixed populations of several species.

This implies that a robust reference analysis has been previously performed. For this purpose, it is crucial to set up a large data base by progressively gathering informations from field collecting. In addition, such a data base would allow to investigate, from a more quantitative point of view, some of the questions previously mentioned: ecological and geographical variability, more accurate description of the distributions obtained from the species of the « *Anopheles maculipennis* complex », composition of mixed populations...

The study of hair branches distributions in the « *Anopheles maculipennis* complex » using CA method, shows that larval chaetotaxy can be an effective taxonomical criterion. Furthermore, the method increases the discriminative effect of this criterion. It permits to control identifications established by the study of eggs, to identify new or litigious strains and to detect mixed populations of several species. Creating a data base, thanks to individuals collected over the Lyon region or over a larger area would increase the precision and the robustness of the analysis. It would also allow to investigate more deeply geographical variation of the chaetotaxic character. However, it remains that the identification of species from « *Anopheles maculipennis* complex » can only be performed on mosquitoes larval progeniture and not on adults. Extensions of the method also result from adding other types of data into the data base, such as isoenzymes characters. The use of analogous computation methods, could then lead to immediate identification of any adult from « *Anopheles maculipennis* complex ».

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