NOTES ON DIFFERENCES BETWEEN SOME EXTERNAL AND SKULL CHARACTERS OF MICROTUS ARVALIS (PALLAS, 1778) AND OF MICROTUS AGRESTIS (LINNAEUS, 1761) FROM THE NETHERLANDS

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CONTENTS

INTRODUCTION

A number of characters has been used by several authors to seperate *Microtus arvalis* (Pallas, 1778) from *Microtus agrestis* (Linnaeus, 1761). Husson (1962: 27-28, 33) enumerated several characters of these forms to identify skull remains from owls pellets found in The Netherlands, Belgium and Luxemburg. Frank (1953) and Van Wijngaarden (1956) gave some external characters useful in field work. Unfortunately, none of these characters separates the two species completely and using them it is therefore

not always possible to identify correctly a specimen at hand. The restricted value of the characters used for the identification of skull remains has already been pointed out by Husson (1962: 47).

It is at the suggestion of Dr. A. M. Husson, that I have undertaken the present study in which the two species *M. arvalis* and *M. agrestis* are critically compared and the various currently used characters evaluated as to their usefulness. The examined material was collected in The Netherlands between 1912 and 1967. It has been sampled from the available material in such a way, that the localities of the studied specimens are as widely dispersed as possible (see fig. 1). The two sexes occur in practically equal numbers and the seasons of catch are rather uniformly represented. The frequency distributions of the various measurements do not deviate much from normal distributions; this has not been illustrated.

Measurements have been carried out with callipers, or with a stereomicroscope (8 ×) with ocular micrometer. Each skull was measured twice, the second measurement being performed one day to two months after the first; the average of the two measurements was used.

In the first place I want to express my gratitude to Dr. A. M. Husson, curator of Mammals of the Rijksmuseum van Natuurlijke Historie, Leiden, who suggested the present study to me and gave me valuable advice and

Microtus arvalis   **Microtus agrestis**
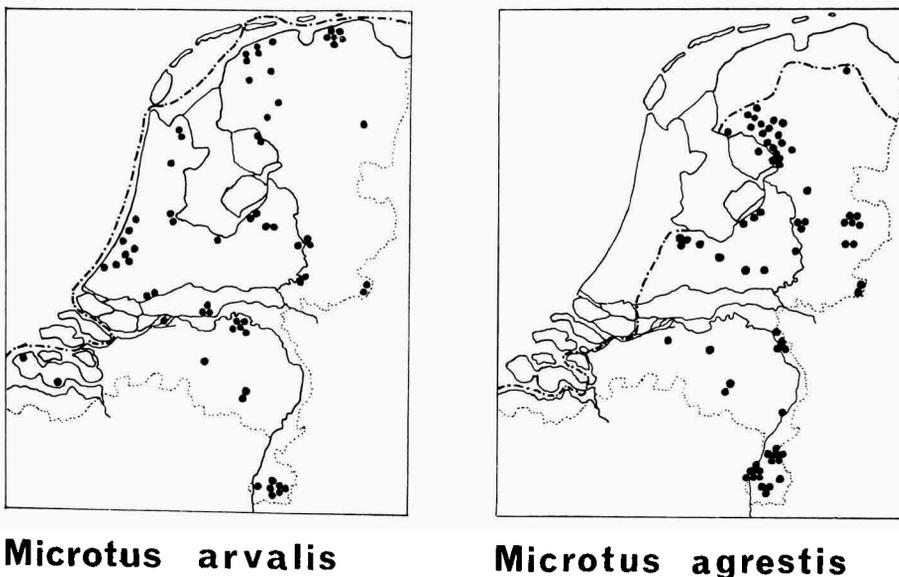
Fig. 1. Maps, indicating the collecting sites of the specimens used in this study. The western limits of the areas of the two species in The Netherlands have been indicated by interrupted lines (after preliminary data of Van Wijngaarden and Van Laar).

help throughout my work. I am much indebted to Mr. P. J. H. van Bree, curator of Mammals of the Zoölogisch Museum, Amsterdam, for his kindness to permit me to examine the material of *M. agrestis* preserved in that museum. Mr. B. Hoekstra at Almelo, The Netherlands, was so kind to send me on loan the specimens of *M. agrestis* of his private collection. It is a pleasure to thank Mr. E. Meelis of the Instituut voor Theoretische Biologie of Leiden University, who has given much help and information concerning the statistical problems. I thank Mr. V. van Laar for communicating the preliminary data, concerning the geographical distribution of *M. arvalis* and *M. agrestis* in The Netherlands, obtained by him in collaboration with Dr. A. van Wijngaarden of the Rijksinstituut voor Veldbiologisch Onderzoek ten behoeve van het Natuurbehoud, Zeist, The Netherlands.

THE IDENTIFICATION OF THE SAMPLED SPECIMENS

During the investigations a total number of 126 specimens of *M. arvalis* and *M. agrestis* was studied. At first this mixed material of the two forms was not identified to species. To arrive at an identification of each specimen a series of measurements were taken, and of some qualitative characters the variability was studied. Most of these measurements and characters were mentioned in the literature; a survey of all studied characters will be given in the following sections.

Not a single of the studied characters can be used by itself to separate the sample indubitably into two or more species. The character which proved to be most useful was the shape of the second upper molar (M^2). As is shown in fig. 2a, this character separates the sample into two well defined groups, but the separation is not complete. It is generally accepted that the left group of the diagram, with five fields at M^2, belongs to *M. agrestis*, and the right group, with four fields, to *M. arvalis*. However, the few specimens between the two peaks cannot be identified, showing that the character is not wholly decisive.

Therefore I looked for other characters to divide the specimens into two similar but more distinct groups. Three characters proved to be useful in this respect, viz. (1) the length of the hairs inserted at the base of the ear and protruding over it (see fig. 3, x_b), (2) the colour of the dorsal surface (see table 2), and (3) the position of the foramen mandibulare (see fig. 12, x_2). The separations that were obtained with these three characters are given in fig. 2b, c, and d. It is clear that none of these separations is complete, no more as this is the case with the separation based on the shape of the second upper molar (fig. 2a).

A closer look showed that the specimens that are situated in the left part
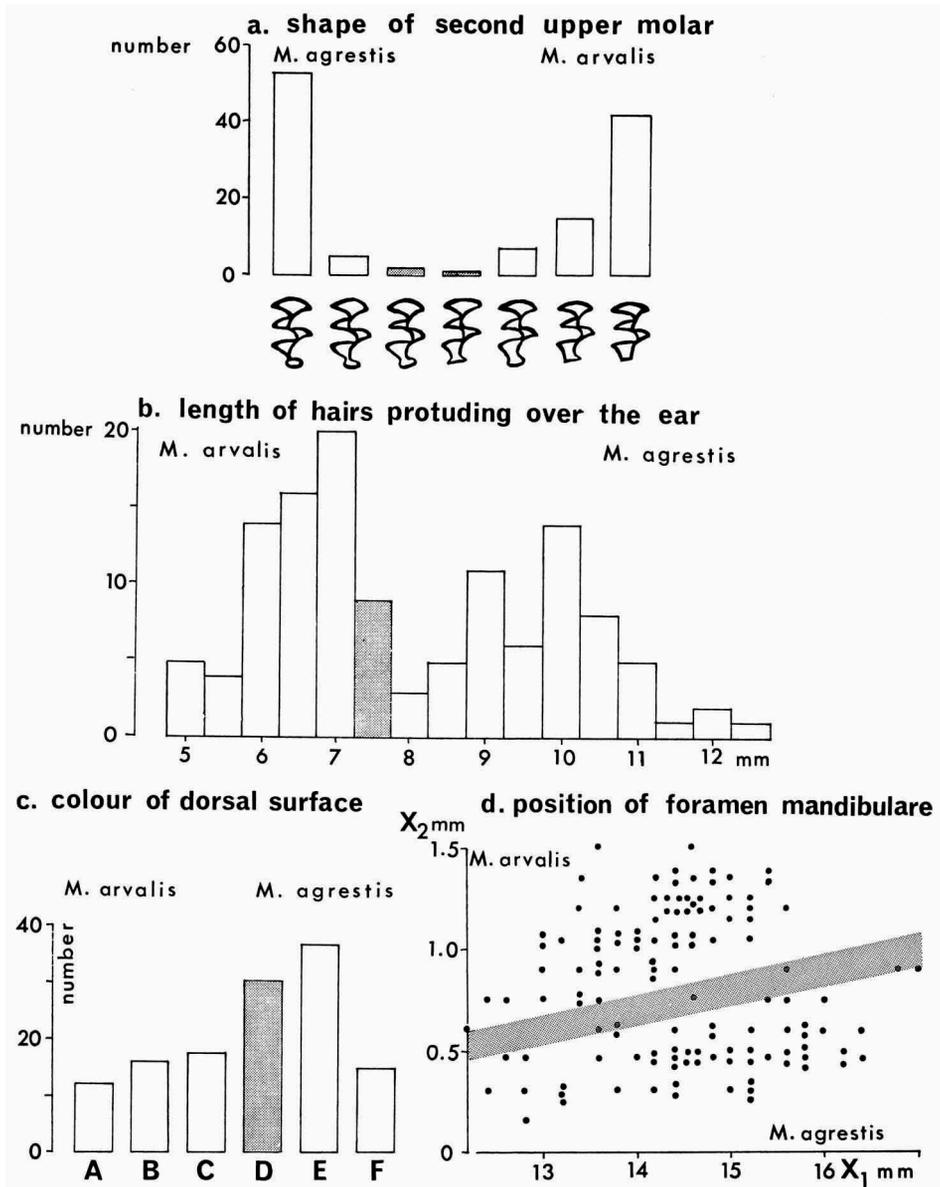
Fig. 2. Frequency distributions (a, b, c) and diagram (d) of the four characters that are used to identify the sampled specimens of *Microtus arvalis* and *Microtus agrestis* from The Netherlands. The grey columns contain data of both species, and separate the white columns which pertain almost exclusively to a single species. The letters A to F in fig. 2c refer to the colour classes given in table 2.

of fig. 2a, mostly occur also in the right part of fig. 2b, in the right part of fig. 2c, and in the lower part of fig. 2d. These specimens consequently belong to one species. Judging by the current views these specimens belong to *M. agrestis*. By the method used in fig. 2a, b, c, and d the specimens are separated into only two groups, while in each case these groups consist of about the same specimens. This indicates that not more than two species are involved: *M. agrestis* and *M. arvalis*. In each diagram it can be indicated which part represents *M. arvalis* and which *M. agrestis*.

Now the question arises where exactly the ranges of the two species must be separated. To this end, it was necessary first to identify the specimens. However, the identification depends entirely on the place where the separation is fixed. To escape from this vicious circle, the identification was executed step by step until the best separation was found. This was considered the most suitable way here to approximate a division into two natural species. While doing this, a separation was assumed at an obvious place, e.g. between two peaks. Then the identification of a specimen was executed as follows. The value of each of the four characters was determined, and it was decided whether it was in favour of either *M. arvalis* or *M. agrestis* according to the assumed separation. If all four characters were in favour of only one species, of course the specimen was decided to belong to that species. If three characters were in favour of one species, while the fourth indicated the other, the identification was based on the three characters. In this preliminary phase it could occur that two of the four characters were in favour of one species, while the other two characters had values of the other species. Such specimens were not identifiable, for the characters were assumed to be equivalent, as their separating ability appeared to be about the same. It is obviously desirable that the number of unidentifiable specimens is minimal, so that the two species can be distinguished as clearly as possible. To achieve this end for each character the point in the range of values, which could be considered the breaking point between the two species, was moved several times until the set of four breaking points was found with which the minimum number of unidentifiable specimens was attained. It proved to be possible to reduce this number to zero. Consequently all specimens were identified in this way, while at the same time it was decided, which values of the four characters belonged to *M. arvalis* or to *M. agrestis*.

When this was achieved, it turned out that in each character some of the values were represented by a number of specimens of both species. These values of course are not useful for identification; they form a "range of incertitude", which area has been indicated with a grey colour in the dia-

grams of fig. 2. This "range of incertitude" was usually smaller than the overlap of the values of the two species, although the difference between them concerned only a few specimens. E.g., the overlap of the character of the second upper molar is as large as the total range of both species together. But when only two specimens are excluded from consideration the overlap shrinks, becoming as large as the "range of incertitude" denoted in fig. 2a. Therefore the extent of the "range of incertitude" is adapted to its practical application.

It was determined for each of the characters which values are characteristic for *M. arvalis*, and which for *M. agrestis*, and also which values are useless for identification. The limits of these ranges are given in fig. 2a to d. It can be easily concluded from this figure that none of the characters can be used by itself for identification, because all show some overlap. So identification must occur with a combination of these characters. This took place by determining for a specimen how many characters were typical for *M. arvalis* (A), or *M. agrestis* (B), and how many characters had a value in the "range of incertitude" (?). In table 1 the frequencies of the possible combinations are given.

TABLE 1

	identifiable						not identifiable		
	AAAA	AAA?	AA??	AAAB	AA?B	A???			
combinations:	+	+	+	+	+	+	AABB	AB??	????
	BBBB	BBB?	BB??	BBBA	BB?A	B???			
% (n=126):	75	14	6	2	2	0	0	0	0

From table 1 can be concluded (a) that all specimens could be identified, and (b) that the specimens that could be identified with great certainty (e.g. the combinations AAAA + BBBB) were more frequent than the specimens that could be less positively identified (e.g. the combinations AA?B + BB?A).

So all studied specimens could be classed in one of the two forms by combining four overlapping characters. Can these forms be considered to be two good species? This is most certain, when is known that the studied specimens are sampled from two reproductively isolated populations. Unfortunately this is not known, but it seems probable that the described differences between the two forms are maintained by isolation. If this is correct, *M. arvalis* and *M. agrestis* are two natural species.

By using the identified specimens, it is possible to verify whether supplemental measurements are useful for identification. This will be done in the next chapter.

Remarks

1. When a character has a value close to its "range of incertitude" (e.g. $x_b = 8.0$ mm, see fig. 2b), it is less likely that this value indicates the correct species, than if the value is situated close to its extreme (e.g. $x_b = 12.0$ mm). Nevertheless in both cases the specimens used for table 1 received a plus sign. No method was used to overcome this disadvantage, as the present method gives satisfactory results.

2. Some other specific differences have been found, additional to the four characters mentioned above. As these differences were only slight, it was not possible to use them at the initial stage of identification of the sampled specimens which has been described before. However, for reasons that will be dealt with in a following chapter, it is yet possible in some way to use them for identification. Consequently the identification in general can be based on more characters than the four mentioned above. See the diagnoses for details.

3. The differences that are dealt with above only concern the structure of the skin, the skull, and the mandible. In the literature other differences are mentioned, concerning the following characters:

a) The chromosomes (see Matthey, 1952: 118, 119).

b) The preferred bottom temperature in a temperature organ. This temperature seems to be higher (35° C.) in *M. arvalis* than in *M. agrestis* (31° C.) (Herter, 1958: 18-19, tab. 1).

c) The habitat. *M. arvalis* often occurs in areas with short grass, while *M. agrestis* mostly occurs in places with a high and dense cover. This is especially clear at localities where both species meet (Frank, 1953: 14; Bernard, 1953; Van Wijngaarden, 1956: 218-219; Reichstein, 1959: 370; personal experience).

d) The voice. According to Frank (1953: 15) *M. arvalis* has a rather high, mostly monosyllabic call, while *M. agrestis* has a more low pitched, mostly polysyllabic call. This is approximately in accordance with my personal experience, but I want to add some remarks. In the first place the mentioned differences are not absolute, so that confusion of the voices is possible. A sound spectogram may show the exact extent of differences. In the second place the two species differ much in the incidence of calling. *M. agrestis* is nearly always noisy when handled, while *M. arvalis* less often uses its voice in these circumstances.

THE VARIABILITY OF THE STUDIED CHARACTERS

1. The external characters

a. The coat colour

It is generally accepted that *M. agrestis* has a dorsal surface which is darker brownish than that of *M. arvalis*. To study the variability of this character the material was divided into six different classes, the members of each class resembling a typically coloured reference individual. The percentage of specimens placed in a colour class was determined for each species; the result is shown in table 2. From this table can be concluded that the classes A, B and C are almost exclusively represented by *M. arvalis*, and the classes E and F by *M. agrestis*. So, when a specimen is coloured like the members of these classes, identification will mostly be correct.

TABLE 2

Variability in colour of the dorsal surface of *Microtus arvalis* and of *Microtus agrestis* from The Netherlands, expressed in percentages of specimens resembling reference individuals. Nomenclature of the colours after Ridgway (1912).

Reg. no. (Rijksmuseum van Nat. Historie) of reference individual		Colour of dorsal surface	% of	% of
M. arva-lis	*M. agres-tis*		*M. arvalis* (n = 68)	*M. agrestis* (n = 70)
6815	-	A. Sardacco's Umber to Sepia	18	0
19006	-	B. Sardacco's Umber to Tawny Olive	24	0
2267(5)	16656	C. Snuff-Brown	24	2
599c	1757	D. Sayal-Brown to Cinnamon-Brown	33	19
-	19014	E. Chestnut-Brown	0	54
6950	19011	F. Mummy-Brown to Sepia	1	24

Class D, on the contrary, is represented by rather large percentages of specimens of both species. So, if a specimen is coloured like these skins, the colour of the dorsal surface is not useful in identification. However, I presume that such a result would not have been obtained if only recently collected material had been used. The specimens of *M. agrestis*, placed in class D (19%), were all collected before 1950. I have studied over 80 specimens of *M. agrestis* collected after 1950 in The Netherlands, partly included in the sample and partly consisting of additional, mostly fresh material, and no specimen of this more recently preserved material showed the colour of class D. Therefore I wonder if the specimens of *M. agrestis* placed in

class D have not changed colour after preservation in the musea. In fresh state these specimens were perhaps coloured like those of class E or F. If this is true class D would be filled up with *M. arvalis* only. So the separation based on the coat colour is possibly much better when only recently collected specimens are studied. However, even then the separation is not perfect.

The conclusion can be drawn that in The Netherlands the dorsal surface of *M. arvalis* is generally greyish brown, yellowish brown, or reddish brown, while specimens of *M. agrestis* are dark grey or dark chestnut brown.

This difference in colour of the dorsal surface is probably connected with the difference in the habitat of the two species. *M. arvalis*, which is often visible from above by birds of prey because of its more open habitat, has in my opinion often a colour resembling that of the substratum of the localities where it is caught. *M. agrestis* is probably darker because of its more covered and thus darker habitat.

b. The length of hairs

It has been noted, e.g. by Frank (1953: 15), that the ears of *M. agrestis* for the greater part are covered by the fur, while those of *M. arvalis* protrude more freely. This proved to be correct, because the hairs of the head that are inserted near the base of the ear are longer in *M. agrestis* than in *M. arvalis*. To study the variability of this character, the length of these hairs (x_b) was measured in the studied specimens. As fig. 3 makes clear, this character separates the species fairly well, although the values of the species slightly overlap. In the overlap range, when $x_b = 7.5$ mm, ear length (x_c) can be used as a supplemental character, specimens with ears shorter than 7 mm belonging to *M. agrestis* and the rest to *M. arvalis*.

Such differences in the length of the hairs were not found on other body parts, e.g. the middle of the dorsal side. In the two species the length of these hairs measured in a small number of specimens were about equal, both being similar to the average of x_b of *M. agrestis*. The differences in hair length are obviously restricted to the hairs inserted at the base of the ear.

c. The length of the meatal lobe of the ear

Several authors, e.g. Miller (1912: 681) and Ognev (1964: 139-140), mentioned that the meatal lobe of the ear (fig. 3, x_a) is longer in *M. agrestis* than in *M. arvalis*. To study this character, the length of the meatal lobe was measured in more than 60 specimens of each species. As the superior edge of this lobe is often not a straight line, like the auricle of *M. agrestis* pictured in fig. 3, a standard for measuring was assumed, being the greatest length of the meatal lobe. The results can be found in fig. 3. In this figure some difference between the species can be noticed. The lobe was maximally 2 mm

long in *M. arvalis*, while it was frequently longer than 2 mm in *M. agrestis*. On the contrary the minimal length of the lobe was similar when the two species were compared. Consequently the greater part of the ranges of the two species overlap. Only 36% of *M. agrestis*, having a lobe longer than 2 mm, would be identifyable with this character. The remainder of the specimens is not identifyable when this character is used by itself.

d. The tail.

Van den Brink (1968: 106) and Bernard (1953 : 3) remarked that there is a difference in the colour of the tail, *M. arvalis* having a light unicoloured tail, and *M. agrestis* a bi-coloured. In my material of both species the colour of the dorsal surface of the tail was about similar to that of the dorsal surface of the animal, while the ventral surface was light grey. In most specimens of each species the dorsal surface of the body was much darker than
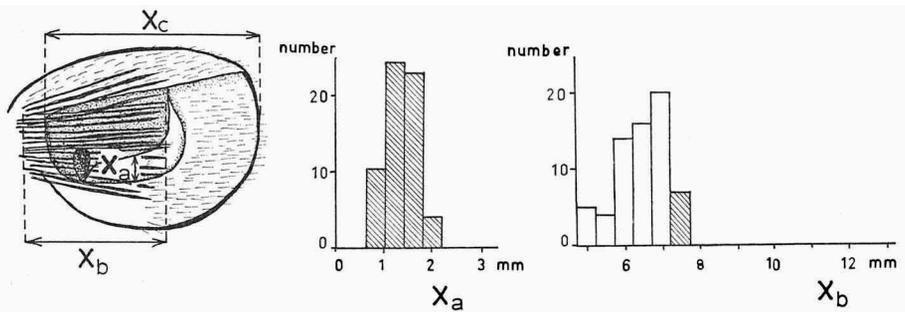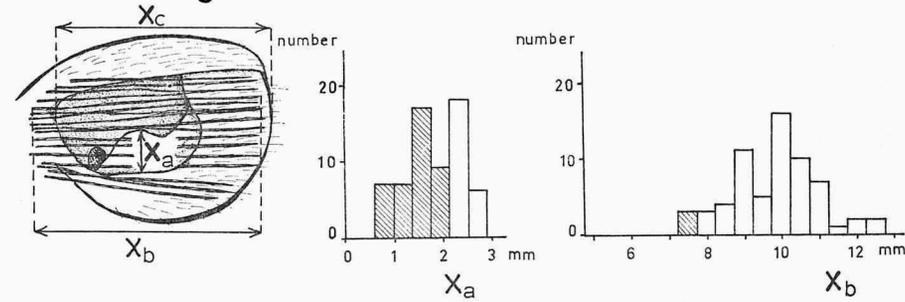
Fig. 3. Measurements of some structures of the ear. The hatched columns indicate the overlapping values of the two species. — x_a: the length of the meatal lobe; x_b: the length of the hairs inserted at the base of the ear and protruding over it; x_c: the length of the auricle.

the ventral surface, consequently most specimens of both species had a bi-coloured tail. Only the few specimens of colour class B (24% of *M. arvalis*, see table 2) were so lightly coloured at the dorsal surface of the entire body, that the tail could be taken as to be unicoloured.

So Van den Brink's statement cannot be considered to form a general rule. Moreover, when a tail of a specimen is unicoloured, the colour of the dorsal surface is light yellowish brown, which is a more obvious difference from *M. agrestis*.

e. The feet

Mohr (1950: 15, 66, fig. 67: 10, 11) stated that the tubercles on the feet were bigger and situated closer together in *M. agrestis* than in *M. arvalis*. Van Wijngaarden (1956: 220) accepted this character for material from The Netherlands. Neither living individuals, nor dry or alcohol preserved specimens seen by me showed a specific difference in this respect. I got the impression that bigger specimens of each species had relatively bigger tubercles.

Ognev (1964: 139) remarks that in *M. agrestis* the proximal tubercle of the hind feet is placed near the edge, while it is situated near the middle in *M. arvalis*. In my material such a specific difference could not be found, the proximal tubercle being situated near the middle of the foot in both species.

I come to the conclusion that only two external characters are generally useful for identification: (1) the colour of the dorsal surface, and (2) the length of the hairs, that are inserted at the base of the ear and protrude over it.

2. The shape of the molars

The molars of the species of the genus *Microtus* have a chewing surface which consists of a number of more or less triangular fields (figs. 4-9). In some species the number of these fields is rather constant, while it varies greatly in others. According to Hinton (1926: 102-124) a decrease of the number of fields in the genus *Microtus* is an evolutionary trend. This means that in phylogeny the percentage of individuals with a smaller number of fields has increased. Reduction of a field proceeds gradually, so that in recent populations all intergradations between a fully present and a completely eliminated loop can be found. This can, e.g., be demonstrated in the second upper molar of *M. agrestis*. The greatest number of loops that has been found in this molar is five (fig. 9x). At first the fifth (posterior) field is as large as the fourth (fig. 9k). Reduction of the fifth field starts with

Microtus arvalis (n=67)

94   3   0   3%

M₁

Fig. 4

a   b   c   d
                      „maskii"

Microtus agrestis (n=67)

90   7   4%

e   f   g

Microtus arvalis

M₂

Fig. 5

a   b   c

Microtus agrestis

d   e   f

Microtus arvalis

M₃

Fig. 6

a   b   c

Microtus agrestis

d   e   f
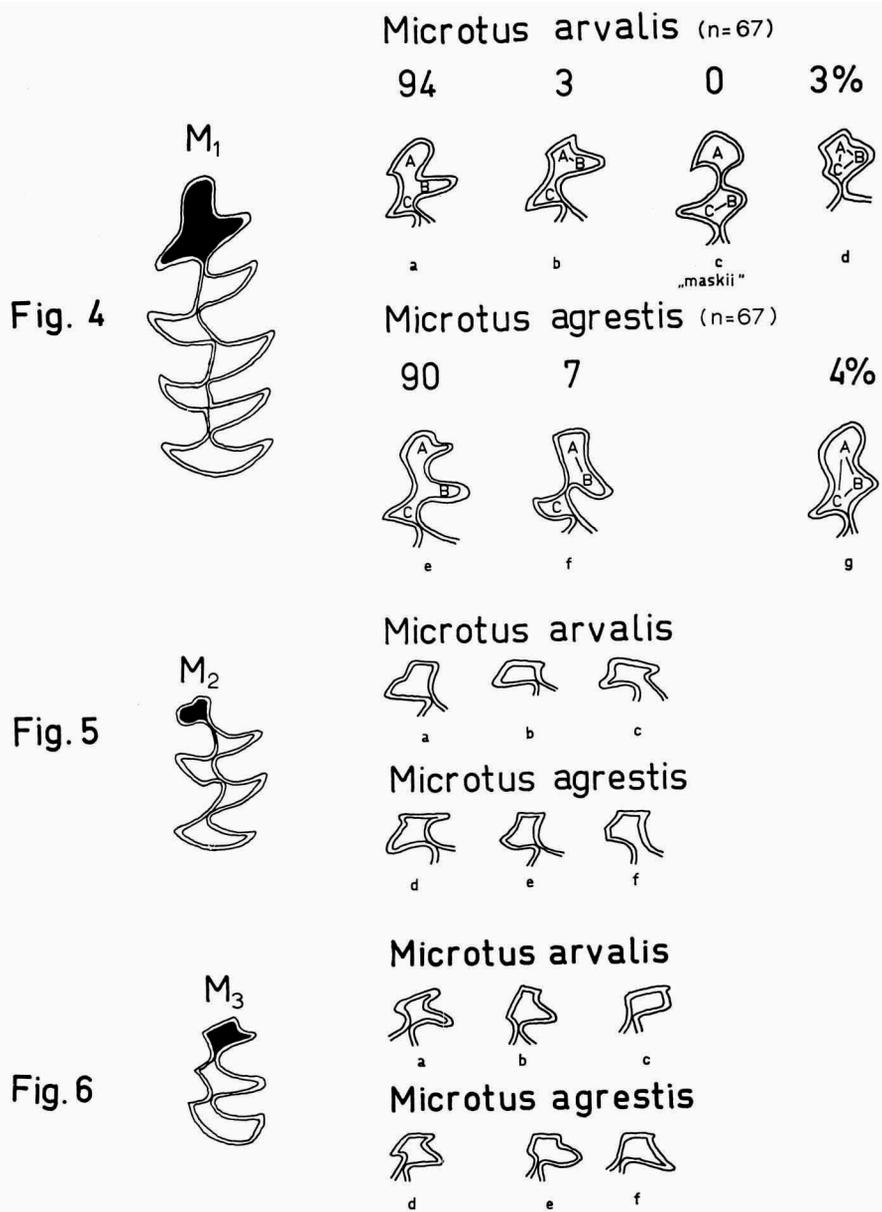
Figs. 4-6. Variations in the structure of the anterior part of the lower molars of *Microtus arvalis* and *Microtus agrestis* from The Netherlands. At the left the entire chewing surfaces are pictured. The variation of the anterior parts (indicated in black in the left hand figures) are given at the right. Similar shapes found in the two species are placed below one another. From the left to the right the number of fields gradually decreases. The percentages indicated in fig. 4 are based on a sample of 67 specimens of each species. — 4a after RMNH no. 705; 4b after no. 5267; 4d after

the loop becoming smaller (fig. 9 l). In the next stages of reduction the separation between the fifth and the fourth field gradually disappears (fig. 9 m, n, p). Together with the disappearance of this separation the fifth field may further decrease in size, when compared with the fourth (fig. 9 o, q). Finally the fifth field is eliminated entirely (fig. 9 r and y). In others molars the process of reduction is principally the same, as can be judged from figs. 4-8.

The process of reduction, which has been described above, applies to both *M. arvalis* and *M. agrestis,* and certainly also to a number of other species. Reduction leads to differences between two species, when it has been advanced further in one species than in the other. How far this has been realised in the molars of *M. arvalis* and *M. agrestis* will be described below.

The mandibular molars of each species show most variations in the shape of the anterior part of the chewing surface. Such variations, found in my material, are pictured in figs. 4-6. When comparing the two species, no important differences can be noticed in these features. However, some details are interesting.

The first mandibular molar of each species is irregular in shape. Three parts, A, B, and C can be discerned (fig. 4). Mostly these parts are not well separated (fig. 4a, e). Sometimes parts A and B are united, while part C is well separated from them (fig. 4b, f). The maskii-form, see e.g. Rörig & Börner (1907: 76, fig. 123), with part A well separated from the united parts B and C, is not present in my material (fig. 4c). In some specimens all three parts are united (fig. 4d, g); this is as far as I know the most strongly reduced form of the molar.

The second and third molar have frequently an anterior field, which is incompletely divided into two parts (fig. 5a, d, fig. 6a, d). In my opinion it is probable that in earlier times these two parts were completely separated, M_2 and M_3 having had one field more than in recent times.

Although the mandibular molars are variable in shape, these variations do not provide obvious differences between *M. arvalis* and *M. agrestis.*

In comparison with the mandibular molars, the upper molars show more specific differences. At first the observed variations in the structure of these

molars will be described; at the end of this chapter the diagnostic value of the upper molars will be given.

The anterior molar, M^1, of *M. agrestis* in some specimens has a distinct sixth field (fig. 7 d, e). This type has been called the exsul-form. The exsul-form is not known to me in *M. arvalis*. Also in populations outside The Netherlands, the exsul-form has been found in some *M. agrestis*, and not in *M. arvalis* (Zimmermann, 1956: 272). However, both species do often have rudiments of the sixth field (fig. 7a, b, f, g). An M^1 with only five fields also occurs in both species (fig. 7c, h), but is more common in *M. arvalis* than in *M. agrestis*.

The second upper molar, M^2, is generally considered to be the most important character to separate the two species, *M. agrestis* is said to have five fields at this molar, and *M. arvalis* four (fig. 9 respectively x and y). However, exceptions to this rule have repeatedly been recorded in literature concerning material from outside The Netherlands. Reichstein & Reise (1965) have given a survey of the variation of M^2 of *M. agrestis*, and mentioned several exceptions to the above rule. Of course such exceptions can only be detected when the identification of the specimens at hand is based on a number of other characters, as is done in the present study.

In my material the shape of the second upper molar proved to be variable in both *M. arvalis* and *M. agrestis*. These variations, supplemented by some data from abroad, have been pictured in fig. 9. After comparison of this character in the two species it becomes evident that there is no qualitative difference here between *M. arvalis* and *M. agrestis*. In contrast to this, there are important differences between the species, with regard to the frequency in which the various molar shapes occur. In *M. arvalis*, specimens with a better or moderately developed fifth field are scarce (fig. 9 a-d: 4% of 67 specimens), while in *M. agrestis* nearly all specimens have five fields (fig. 9 k-o: 99% of 67 specimens of this species). Specimens with inconspicuous rudiments of the fifth field are common in *M. arvalis* (fig. 9 e-f: 34%), but such are absent in the sampled material of *M. agrestis* (the pictured molar parts of fig. 9 p and q are from specimens from abroad). Specimens with only four fields are very common in *M. arvalis* (fig. 9 g: 61%), while only one of my specimens of *M. agrestis*, a juvenile, showed four fields (RMNH no. 4391). Other juveniles showed five fields.

The posterior upper molar, M^3, may have five, four or three inner loops (fig. 8 respectively d, e and c). The form with three inner loops has been called simplex by Rörig & Börner (1907: 72). In *M. agrestis* four inner loops are normal. According to Reichstein & Reise (1965: 38-39) in this species the simplex-form has only been reported from Denmark. In 175 skulls
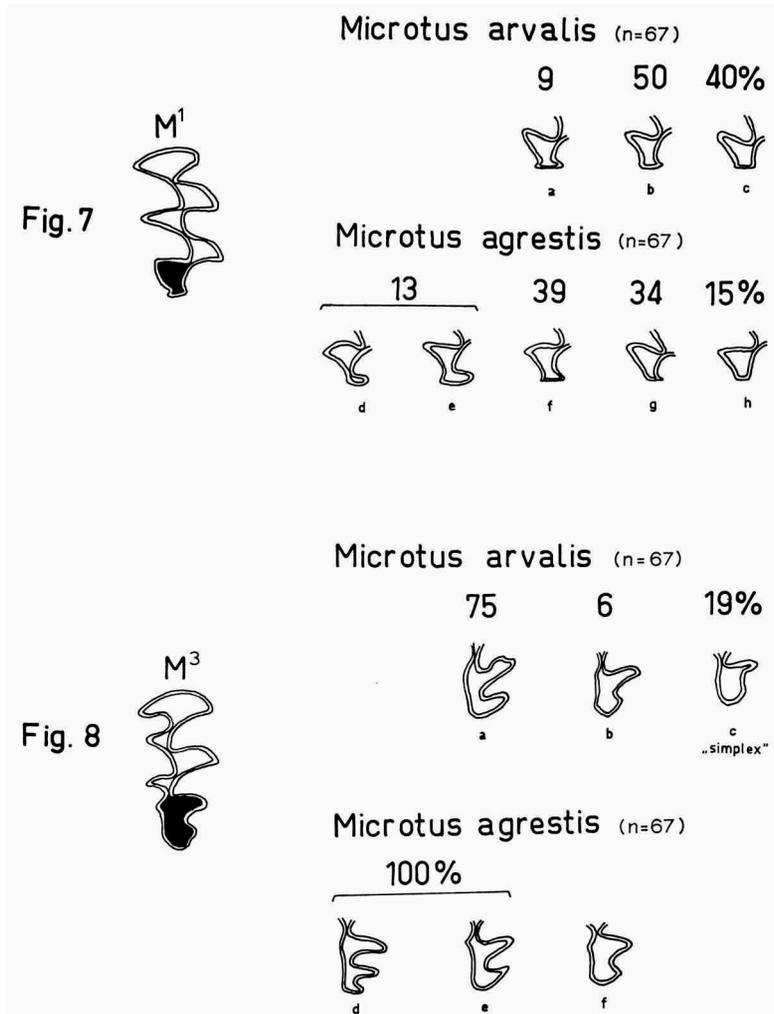
Figs. 7 and 8. Variations in the structure of the posterior part of the first and third upper molar, M¹ and M³, of *Microtus arvalis* and *Microtus agrestis*. At the left the entire chewing surfaces are pictured. The variations of its posterior part (indicated in black in the left hand figures) are given on the right. Shapes that are similar in the two species, are placed below one another. From the left to the right the number of fields gradually decreases. The percentages indicated are based on a sample of 67 specimens of each species. The specimen of fig. 8f, with 0%, did not occur in these samples, but has been found in other material. All figures after right molars. — 7a after no. 19267; 7b after no. 19015; 7c after no. 19220; 7d after no. 19019; 7e after no. 4547; 7f after no. 5077; 7 g after no. 16103; 7h after no. 3563; 8a after no. 632(3); 8b after no. 1113c; 8c after no. 3563; 8d after no. 5217; 8e after no. 1360; 8f after a specimen from Bergen op Zoom, The Netherlands.
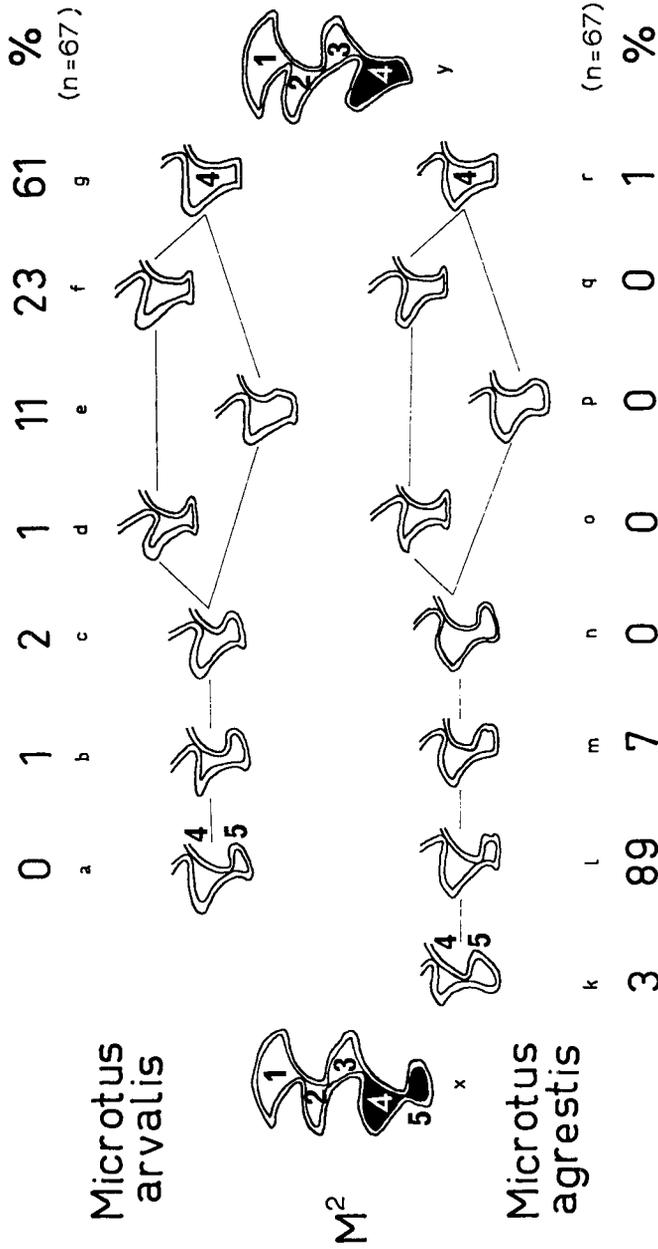
Fig. 9. Variations in the structure of the posterior part of the second upper molar (M^2) of *Microtus arvalis* and *Microtus agrestis*. At the left (x) an entire chewing surface with five fields is pictured, at the right (y) one with four fields. The variations of its posterior field are given in between. Variations of both species that are similar, are placed below one another. From the left to the right the fifth posterior field gradually disappears. The percentages are based on a sample of 67 specimens of each species. The specimens of figs. 9a, n, o, p, and q, with 0%, did not occur in these samples, but are found in other material or in the literature. All represent right molars. — The following figures are after specimens of the Rijksmuseum van Natuurlijke Historie, Leiden: a after no. 6028; b after no. 6297; c after no. 6929; d after no. 732(3); e after no. 5402; f after no. 5078; g after no. 6504; m after no. 19012; n after no. 19011; o after no. 19011; k after a specimen from Hoekstra's collection, number 659. These specimens are all from The Netherlands. The following figures are after specimens from Denmark: p after Reichstein & Reise (1965, fig. 1k), q after Ursin (1948, fig. 1d), and r after Winge (1875, fig. 5).

of *M. agrestis* from The Netherlands examined by me the simplex-form has not been discovered. In only one skull a slightly reduced fourth inner loop occurred (fig. 8 f), indicating in my opinion that the tendency of disappearance of this loop is not entirely absent in The Netherlands.

In *M. arvalis* the simplex-form is more common. The incidence of this form differs locally. To demonstrate this I roughly divided The Netherlands into three regions with markedly different percentages of simplex-forms (fig. 10). As a measure of differences between the studied populations the test statistic of the χ^2-test was computed. This test statistic had high values for the relations between the populations of different regions, which indicates relatively great differences between them. On the other hand, its values of the relations between populations within the regions were small, which indicates similarity. These results do not imply that the proposed subdivision is the only correct one, but merely indicate an amount of difference between the regions, and a relative homogeneity within the regions.

The simplex-character of *M. arvalis* has been studied by a number of authors. Their results and discussions can be summarized as follows:

a) Two concentrations of the simplex-form of *M. arvalis* are known, one in Southern Jutland and one in Russia. In the remaining part of the areal of the species the incidence of the character is less than 5% (Zimmermann, 1952: 494, fig. 3).

b) In these two concentrations the incidence is highest close to the boundary of the range of the species. The incidence gradually decreases when radiating from these regions (Zimmermann, 1935: 260, fig. 2; 1952: 494, fig. 3).

c) The normal form of the third inner molar (with four inner loops) is partly dominant over the simplex-form. Inbreeding produces a greater number of simplex-forms than the Mendelian laws predict (Zimmermann, 1952: 495; 1958: 39).

d) The causes of the regional differences in the incidence of the simplex-form are quite obscure. Stein (1958) and Zimmermann (1958) have discussed the influence of selection. No selection has been proved in this respect. Another process, genetic drift, is unlikely here, since the populations are large and genetic drift will not cause a clinal decrease of incidence from a centre. Diffusion of the character from its marginally situated concentrations may be a possible cause.

With respect to The Netherlands some remarks may be made:

a) The relatively high incidence of the character in the Northern and Eastern part of the country is connected with the concentration of simplex-forms in Denmark and Germany.

b) There are no important correlations between incidence and soil or climate.

Fig. 10. Geographic variation in the incidence of the simplex-form (see fig. 8c) of
Microtus arvalis in The Netherlands. Three roughly delimited regions are recognized,
that have markedly different incidences of this form. The figures on the map cor-
respond with the number of studied specimens from these localities. From the regions
marked with an A no material was available; the species is not known from the islands
that are left white.

The larger rivers do not seem to be barriers. Consequently these factors do not give an explanation for the distribution of the simplex-character. Other causal factors are not obvious.

c) Two regions in which *M. arvalis* invaded after previously being absent have been studied, viz. the North Sea island Ameland and the Noordoost-polder. In these two parts the incidence of the simplex-form was similar to that of the regions from which the populations originated. Apparently invasion and adaptation to a new environment have not changed the incidence. In my opinion the exact distribution of the simplex-form may reveal interesting information.

The diagnostic value of the three upper molars will be described now. The variations of the shapes of these molars and their frequencies, found in the material of *M. arvalis* and *M. agrestis* studied by me, are given in fig. 11. The first upper molar may have six or five fields, the second upper molar five or four, and the third upper molar six or five (see fig. 11 M^1, M^2, and M^3). Each molar shows two types: a "complicated" form that has one

	M^1		M^2		M^3		a	b	c	d	e	f	g	h
% Microtus arvalis (n=67)	0	100	4	96	81	19	0	5	73	22	0	0	0	0
% Microtus agrestis (n=67)	13	87	99	1	100	0	13	85	1	0	0	0	0	0
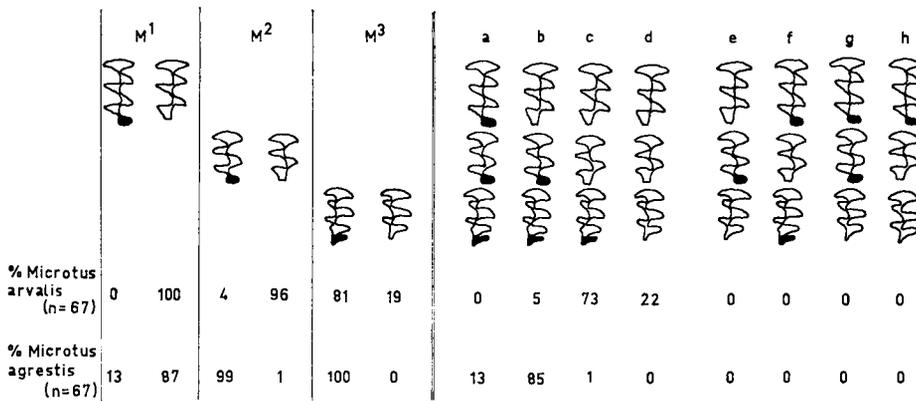
Fig. 11. Percentages of specimens of *Microtus arvalis* and *Microtus agrestis* from The Netherlands, with certain shapes of the three upper molars. Each molar may have principally two shapes, one of them having one field more (which is indicated in black) than the other. Left: variations and incidence of the several molar shapes separately. Right: incidence of the theoretical possibilities with which the molar shapes can be combined in one jaw.

field more than the "simplified" form. The two species differ much in the frequencies in which some of these molar forms occur.

As for M^1 the complicated form only occurs in *M. agrestis*, while the simplified form is common in either of the two species. Consequently this molar can only be used for identication if it has the complicated shape with six

fields, specimens with such a shape belong to *M. agrestis*. Not more than 13% of this species could be identified with this character.

As for M² the complicated form is characteristic for *M. agrestis*, but proved to be not entirely absent in *M. arvalis* (4%). The simplified form is characteristic for *M. arvalis,* and was found in only one specimen of *M. agrestis*. Consequently the shape of this molar is a very important specific character, as has often been emphasized in the literature, but the character is not completely decisive.

As for M³ the complicated form is common in either species, while the simplified form occurs exclusively in *M. arvalis*. Consequently this molar can only be used for identification when it has the simplified form with five fields, specimens with such a shape belong to *M. arvalis*. Not more than 19% of this species could be identified with this character.

It is evident that *M. agrestis* has the greater number of fields when compared with *M. arvalis*. Therefore, in this respect, *M. agrestis* must be considered the most primitive of the two species, since evolution goes from complicated to simplified (Zimmermann, 1956).

Having paid attention to the upper molars separately, we now may consider how in my material the various molar forms are combined in one jaw. This could be important for the identification, for when the molar forms are combined with a high degree of interdepenence, the identification based on the three molars is not more useful than that based on only one of them. To test whether the molar forms of one jaw are combined independently, the expectation of a particular set of three molar forms was calculated by multiplying the chances for each of the three forms. The expectations for all combinations calculated in this way were compared with the values actually found, that are presented in fig. 11 a to h. The calculated and observed values agreed very well. Consequently we cannot reject the hypothesis, that the various molar forms combine independently in one jaw. This means that the results of the identification, based on the three molars separately, remain decisive when these are considered together. Another conclusion is, that the assortment of the genes, corresponding with the forms of the three molars, is obviously independent.

3. The characters of the skull (without mandibles)

With only a skull of either of the two species at hand, the identification was usually based on the shape of the second upper molar. However, from the above mentioned data it is clear that this character is not absolutely reliable. Therefore other characters of the skull have to be looked for, and to this end a number of measurements was taken. These measurements are:

(a) diastema length, (b) rostral height, (c) interorbital breadth (all measured in accordance with Husson, 1962: 12-20), (d) distance between the inner edges of the molars, (e) palatal length minus the distance between the posterior edge of the foramen incisivum and the anterior edge of the incisivi, (f) palatal length, (g) distance between the anterior edge of the foramen incisivum and the anterior edge of the incisivi, (h) length of the foramen incisivum, (i) breadth of the foramen incisivum and (j) length of the upper molar row. One part of these measurements, viz. a to e, proved to be useless for identification. The other measurements (f to j, see fig. 12, x_3 to x_7) separated the species to some degree when plotted in diagrams. These diagrams are not given here. All of these five measurements have a large overlap in my material (see table 3). Consequently it is not practical to use them by themselves and therefore they have been combined with a statistical method, called the discriminant analysis. The principles of the discriminant analysis are dealt with by Anderson (1958). Bühler (1964) has given an

TABLE 3

Measurements of the skull and the mandible of *Microtus arvalis* and *Microtus agrestis* from The Netherlands (in mm).

measurement (see fig. 12)	*Microtus arvalis* (n = 68) mean	range	*Microtus agrestis* (n = 58) mean	range
x_1 mandibular length	14.1	12.2-15.4	14.7	12.4-17.0
x_2 position of foramen mandibulare	1.1	0.6- 1.5	0.5	0.2- 0.9
x_3 palatal length	12.9	10.2-14.4	13.2	10.6-15.0
x_4 incisivi to foramen incisivum	3.6	2.8- 4.4	3.2	2.2- 4.0
x_5 length of foramen incisivum	4.1	3.3- 5.1	4.6	3.3- 5.3
x_6 breadth of foramen incisivum	1.1	0.8- 1.4	1.2	1.1- 1.6
x_7 length of upper molar row	5.6	4.8- 6.2	6.1	4.5- 6.9

application of this method in the field of zoology. A discriminant function can only be developed when the specimens are identified previously, which is the case here. The necessary computations have been carried out on the computer of the Centraal Reken Instituut of the University of Leiden, with an I.B.M.-program described in "System 360, Scientific Subroutine Package, Programmers Manual", which program is based on Dixon (1964) and Anderson (1958). These computations resulted in the discriminant function $X_8 = -0.336\,x_3 + 1.647\,x_4 - 0.057\,x_5 - 1.100\,x_6 - 0.769\,x_7 + 4.778$. The value of X_8 has been computed for each specimen. The frequency distribution diagram of X_8 is given in fig. 12. From this figure can be read that
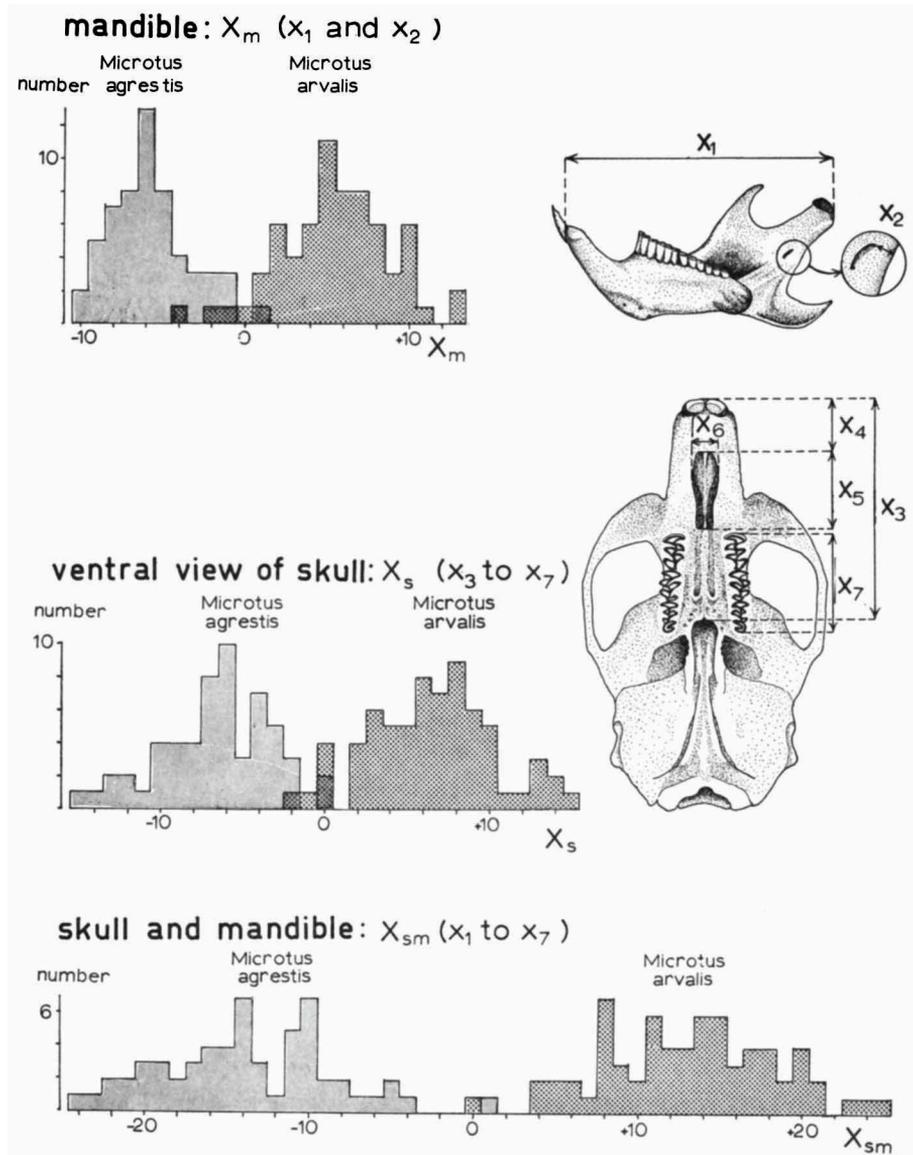
Fg. 12. Measurements of the mandble and the skull of *Microtus arvalis* and *Microtus agrestis* from The Netherlands. X_m: discriminant function which combines the measurements x_1 and x_2 of the mandible; X_s: discriminant function which combines the measurements x_3, x_4, x_5, x_6, and x_7 of the skull; X_{sm} discriminant function which combines the measurements x_1 to x_7 of both the mandible and the skull. The values of these discriminant functions are computed for the sampled specimens, and its frequency distributions are given.

the two species can be rather well separated with X_s, but some overlap is present.

4. The characters of the mandibles

In order to find specific differences in the mandibles of *M. arvalis* and *M. agrestis*, the following measurements have been taken at either the right or the left mandible of the sampled specimens: (a) mandibular length, (b) distance from the posterior edge of the foramen mandibulare to the posterior edge of the mandible, (c) length of the molar row, and (d) height of the processus condylicus. Two of these measurements proved to be useful for identification, viz. the mandibular length (fig. 12, x_1), and the distance from the foramen mandibulare to the posterior edge of the mandible (fig. 12, x_2). When these two measurements are plotted in a diagram two groups are discernable (fig. 2d). These measurements have also been combined with the discriminant analysis, not to check the diagnostic value of x_1 and x_2, which would be incorrect as these measurements are used for the prior identification of the specimens, but merely for practical use. This resulted in the function $X_m = -0.117 x_1 + 1.804 x_2 + 0.277$. The values of X_m have been computed for each of the sampled specimens and are given in fig. 12. It is clear that the function separates the species, but there is some overlap, like in the scatter-diagram of fig. 2d. When the boundaries of the overlap are fixed arbitrarely at $X_m = -2$ and $X_m = +1$, 10% of the sampled mandibles would be unidentifiable. No other distinguishing characters of the mandibles have been found so that the aforementioned results could not be improved upon.

After studying a number of other specimens of *M. arvalis*, from only a few localities, it appeared that many of the small mandibles (shorter than 13.0 mm) would be identified as *M. agrestis* if X_m is used. Consequently very small mandibles are not identifiable when no other fragments of the specimens are available.

Krommenhoek & Slob (1967) have also tried to test the practical value of some mandibular characters. They used mandibles from owl pellets from only two localities in The Netherlands. These mandibles could not be identified individually, since the rest of the animal was of course not available. Consequently Krommenhoek & Slob were obliged to identify their mandibles with the same characters of which they wanted to test the usefulness for identification. This obviously cannot be correct. The extent of the overlap, and consequently the usefulness of a character, can only be estimated when the studied specimens are identified with a greater number of characters.

5. Skull with mandibles

If both the skull and a mandible are available, the measurements x_1 to x_7 (see table 3 and fig. 12) can be taken. These measurements have been used for the computation of another discriminant function, $X_{sm} = 0.04\,x_1 + 2.59\,x_2 - 0.52\,x_3 + 1.14\,x_4 - 0.24\,x_5 - 1.13\,x_6 - 0.63\,x_7 + 6.27$. The values of this function have been computed for each of the sampled specimens. The results can be found in fig. 12. It is clear that this function separates the two species almost without overlap. It is the best distinguishing character to be found during this study, but also the most time consuming one.

6. Skin with skull and mandibles

When of a specimen the skin, the skull, as well as a mandible are at hand, it can most easily be identified with the following four characters: (a) the colour of the dorsal surface, (b) the length of the hairs at the base of the ear, (c) the shape of the second upper molar, and (d) the position of the foramen mandibulare (X_m). When these four characters all indicate the same species, the identification can be relied upon. However, in cases of doubt, it is better to compute X_{sm}, so that all available information is used. When X_{sm} was used in combination with the characters mentioned at (a), (b), and (c), out of the 126 studied specimens 82% had four characters that all indicated the same species. In 12% three of the characters indicated the same species, while the remaining character either did not allow a decision, or indicated the other species. In 6% of the studied specimens only two characters indicated the same species. The other two characters either did both not allow a decision, or only one character did so while the fourth character indicated the other species. Consequently all studied specimens could be identified, but the occurrence of unidentifiable specimens, in which both species are indicated by equal numbers of characters, seems to be possible. However, in general the identification of *M. arvalis* and *M. agrestis*, based on the four mentioned characters, will be practicable.

DIAGNOSES OF MICROTUS ARVALIS AND MICROTUS AGRESTIS

To identify specimens of these two species the following discriminant functions may be used:

(a) For the skull (without mandible):

$X_s = -0.336\,x_3 + 1.647\,x_4 - 0.057\,x_5 - 1.100\,x_6 - 0.769\,x_7 + 4.778$.

(b) For the mandible:

$X_m = -0.117\,x_1 + 1.804\,x_2 + 0.277$.

(c) For the skull and the mandible together:

$X_{sm} = 0.04 \, x_1 + 2.59 \, x_2 - 0.52 \, x_3 + 1.14 \, x_4 - 0.24 \, x_5 - 1.13 \, x_6 - 0.63 \, x_7 + 6.27$.

x_1 to x_7 (see fig. 12 for explanation) are measured to the tenth of a millimetre. The more the discriminant function differs from zero, the more certain it is that the identification is correct.

Microtus arvalis (Pallas)

Skin: Colour of the dorsal surface greyish brown, yellowish brown, or reddish brown (table 2, A, B and C). Hairs that are inserted at the base of the ear and protrude over it shorter than 7.5 mm (fig. 3, x_b).
Skull: The second upper molar generally with four fields (fig. 9y). $X_s > + 2.000$.
Mandible: $X_m > + 1.000$.
Skull and mandible: $X_{sm} > + 2.00$.

Microtus agrestis (L.)

Skin: Colour of dorsal surface dark chestnut brown or dark grey (table 2, E and F). Hairs that are inserted at the base of the ear and protrude over it longer than 7.5 mm (fig. 3, x_b).
Skull: The second upper molar generally with five fields (fig. 9x). $X_s < - 2.000$.
Mandible: $X_m < - 2.000$.
Skull and mandible: $X_{sm} < - 2.00$.

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