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Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae), I.

Electrophoretic studies on esterase and phosphoglucose isomerase of *Bulinus truncatus*. Organ distribution, geographical variation and taxonomic implications.

By

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With 3 figures.

Summary In the present investigation variation in phosphoglucose isomerase and digestive gland esterases of 50 populations of *Bulinus truncatus* have been surveyed. The area covered by the samples corresponds roughly to the total known distribution area of *B. truncatus* in the Mediterranean Region and Africa. In phosphoglucose isomerase a single band of identical mobility was observed in most samples. Two of the samples were variant and showed identical triplebanded stuctures. No segregation of these three bands were observed. In digestive gland esterases normally a double banded structure was observed. The mobility of one of the bands showed differences in a geographical pattern which supports the morphological division of *B. truncatus* in an eastern and a western group. Within the eastern group a division into the subspecies *B. truncatus truncatus* (AUDOUIN), *B. truncatus sericinus* (JICKELI) and *B. truncatus rivularis* (PHILIPPI) is discussed. In the western group no division in subspecies is indicated. *Bulinus contortus* is regarded as an infraspecific form of *B. truncatus*. It is suggested that the western group of *B. truncatus* is divided into two subspecies, *B. truncatus contortus* (MICHAUD) (north of the Sahara) and *B. truncatus rohlfsi* (CLESSIN) (south of the Sahara). The need for further material to support these preliminary conclusions is fully recognised.

Introduction.

One of the most studied genera of African freshwater snails from a taxonomical point of view is the *Bulinus*. The main reason for the great interest in this genus is that some of the species act as intermediate hosts for *Schistosoma* parasites, which cause bilharzia in man and his live stock. However due to the great variability of the habitats of the snails a very great variation is encountered in morphological and anatomical characters. In order to improve the determination of the species a search for new characters has been going on for some years. These new characters include chromosome numbers and electrophoretic pattern of enzymes and defined proteins.

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Chromosome numbers have been useful in distinguishing between some of the species as the majority of *Bulinus* species have 2n = 36, but a few polyploid species with 2n = 72, 108 respectively 144 are known to occur (BROWN & WRIGHT 1972, BROWN 1976). Of these polyploid species only members of the tetraploid (2n = 72) group are more widely distributed. This group includes one of the important intermediate hosts of *Schistosoma haematobium* and *S. bovis*: *Bulinus truncatus*.

In the electrophoretic investigations esterases have been much used (BURCH & LINDSAY 1967, WRIGHT, FILE & ROSS 1966, WRIGHT & FILE 1968, COLES 1970), but only with limited success (WRIGHT 1971). Contrary to this electrophoresis of egg-proteins (WRIGHT & ROSS 1965, 1966) have been useful in elucidating some of the problems encountered. COLES (1969a, b) has undertaken an electrophoretic survey of 14 enzymes of different species of African freshwater snails including *Bulinus africanus* and *nasutus*. His aim was not at improving the taxonomical characters, and he had to use tissues from several specimens to obtain the electrophoretic patterns. With the aim of improving species discrimination within the genus *Bulinus* electrophoretic methods for single individuals have been worked out at this laboratory, and until now more than ten enzymes are routinely surveyed for their usefulness in taxonomic work with this troublesome genus.

Bulinus truncatus (AUDOUIN) has on a morphological basis been grouped into a number of subspecies of which *truncatus*, *rohlfsi* (CLESSIN), *trigonus* (MARTENS) and *sericinus* (JICKELI) are widespread and accepted. All the subspecies are however unified by having arrowhead shaped mesocone of the first lateral tooth and by having 36 chromosome pairs. However, recently *truncatus*-like *Bulinus* samples with 18 chromosome pairs and susceptible to *Schistosoma haematobium* from North Africa have appeared (LO et al. 1970, MANDAHL-BARTH et al. 1976, FRANDSEN 1977). These are found partly outside the distribution area of tetraploid *truncatus* and may represent the diploid ancestor of the tetraploid *truncatus*.

The present paper describes organ distribution and geographical variation of esterases and phosphoglucose isomerase of *Bulinus truncatus* with 36 chromosome pairs and discusses the taxonomic implications of the observed patterns.

Material and Methods.

The origin of the different stocks surveyed are given in table 1 and figure 1. The wild populations are indicated by w in table 1. The snails were maintained in aquaria as described by FRANDSEN (1976). Chromosome numbers were determined on at least 4 different egg-masses from each of the stocks using a slight modification of the method of CLAUGHER (1971).

Preparation of samples for electrophoresis followed the procedure described by JELNES (1971) using 100 µl water for a snail of 7 mm shell height. Electrophoresis was performed at 2° C in 10% starch and a TRIS-EDTA-Boric acid buffer of pH 8·1. Samples were applied with Whatmann no. 1 chromatography paper. Duration of the electrophoresis was 3·5 hours at 320 V and 45 mA. At the end of the electrophoresis the gel was sliced horisontally and detection of phosphoglucose isomerase activity was made on the lower half of the gel

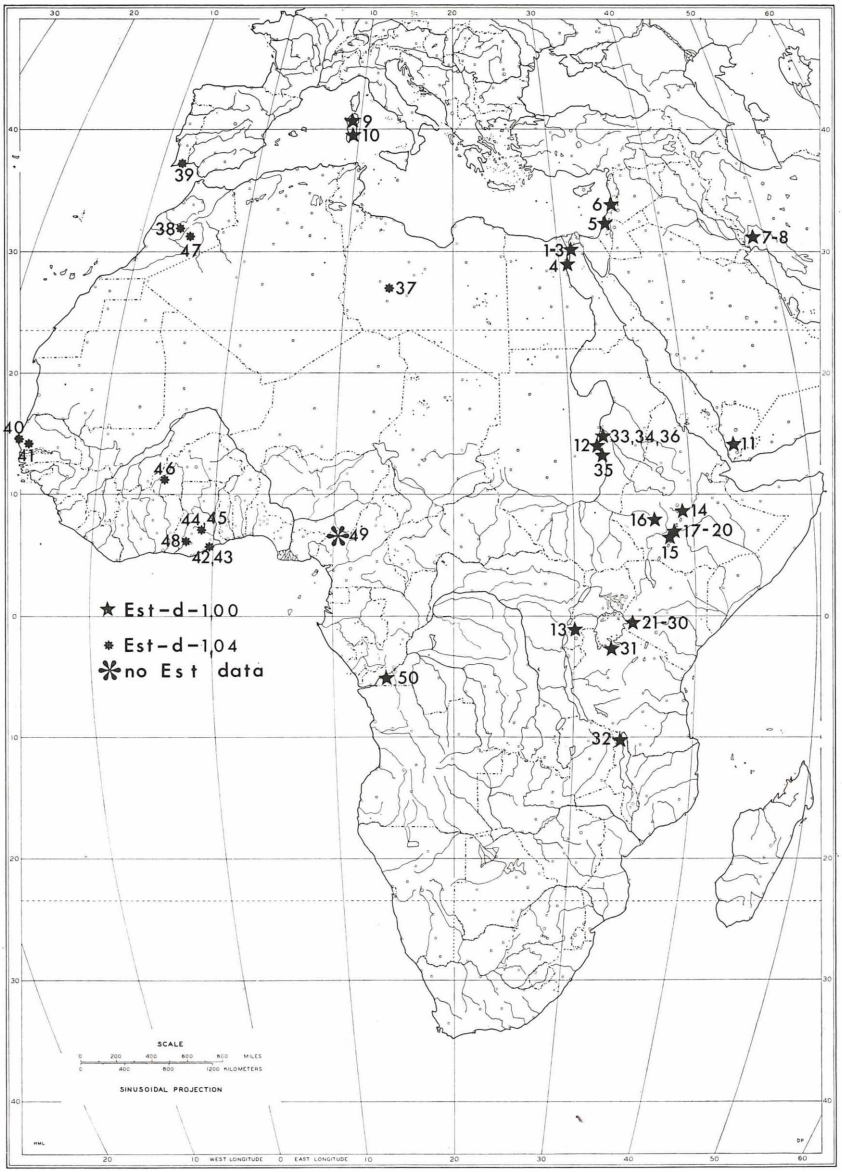

Fig. 1. Map showing the geographical distribution of the samples.

as described by JELNES (1974). Esterase activity was detected on the upperhalf of the gel by a mixture of 250 ml phosphate buffer pH = 7·3 (SICK 1965), 2 ml 1% α-naphthyl acetate in acetone and 100 mg Fast Red TR salt.

Table 1. List of *Bulinus truncatus* (2n = 72) populations investigated. W indicates samples where wild-caught have been tested, see table 2. F indicates samples where only 2-3 laboratory bred specimens have been used. For typical respecively variant enzyme patterns observed in the samples the abbreviations T and V are used.

Number	Subspecies	Locality	Country	Collected	Received from	Est-d	Pgi
1	*truncatus*	Cairo	Egypt	1972	ANDERSEN	T	T
2	*truncatus*	Imbaba	Egypt	1971	NASR	T	T
3	*truncatus*	Giza	Egypt	1969	MANDAHL-BARTH	T	T
4 W	*truncatus*	El-Bashari	Egypt	1976	DEMIAN	T, V	T
5	*truncatus*	Hedera	Israel	1965	WRIGHT (1)	T	T
6	*truncatus*	Sayda-Sur Area	Lebanon	1965	KHALIL-RIF	V	T
7	*truncatus*	Dezful	Iran	1967	MASSOUD	V	T
8 W	*truncatus*	Dezful	Iran	1977	MASSOUD	V	T
9	*rivularis*	San teodora river	Sardinia	1970	WRIGHT (185)	T	T
10	*rivularis*	Posada river	Sardinia	1969	WRIGHT (171)	T	T
11	*sericinus*	Taiz	Yemen	1970	BIOCCA	T	T
12 F	*sericinus*	El Duein	Sudan	1975	WRIGHT (395)	T	T
13	*sericinus*	Lake Mutanda	Uganda	1965	WRIGHT (53)	T	T
14 F	*sericinus*	Wonji	Ethiopia	1969	WRIGHT (245)	T	T
15 F	*sericinus*	Lake Margherita	Ethiopia	1969	WRIGHT (244)	T	T
16 F	*sericinus*	Jimma	Ethiopia	1969	WRIGHT (241)	T	T
17 F	*sericinus*	Adowa	Ethiopia	1969	WRIGHT (237)	T	T
18	*sericinus*	Lake Awasa	Ethiopia	1973	GRABER	T	T
19 W	*sericinus*	Lake Hora	Ethiopia	1975	GRABER	T	T
20 W	*sericinus*	Black River	Ethiopia	1975	GRABER	T	T
21 W	*sericinus*	Rabour	Kenya	1975	BROWN	T	T
22 W	*sericinus*	Otho Market	Kenya	1976	BROWN	T	T
23 F	*sericinus*	Ahero 1	Kenya	1974	WRIGHT (356)	T	T
24 F	*sericinus*	Ahero 2	Kenya	1971	WRIGHT (214)	T	T
25 F	*sericinus*	Rodi Kopany	Kenya	1975	WRIGHT (372)	T	T
26 F	*sericinus*	Chemelil	Kenya	1973	WRIGHT (348)	T	T
27 F	*sericinus*	Ondiek	Kenya	1975	WRIGHT (368)	T	T

28 F	*sericinus*	Kisumu	Kenya	1972	WRIGHT (309)	T	T
29 F	*sericinus*	Lielango River	Kenya	1972	WRIGHT (312)	T	T
30 F	*sericinus*	Kibigori	Kenya	1973	WRIGHT (323)	T	T
31	*sericinus*	Nyanguge	Tanzania	1971	RASMUSSEN	T	T
32	*sericinus*	Karonga	Malawi	1973	DAZO	T	T
33 W	*sericinus*	Gezira	Sudan	1975	SULAIMAN	V	T
34 F	*sericinus*	El Galgala	Sudan	1972	WRIGHT (254)	V	T
35 F	*sericinus*	Kosti	Sudan	1974	WRIGHT (296)	V	T
36	*sericinus*	Husahisa	Sudan	1977	SIRAQ	V	T
37	*contortus*	Sebha	Libya	1969	WRIGHT (166)	T	V
38	*contortus*	Gnafda	Morocco	1967	CORNU	T	T
39 W	*contortus*	Algarve	Portugal	1976	DE LOURDES SILVA	T	T
40	*rohlfsi*	Dakar	Senegal	1974	DIALLO	T	T
41	*rohlfsi*	?	Senegal ?	?	PICOT	T	T
42	*rohlfsi*	Accra	Ghana	1972	ANTESON	T	T
43 F	*rohlfsi*	Akuase	Ghana	1971	WRIGHT (209)	T	T
44 F	*rohlfsi*	Anyaboni	Ghana	1975	WRIGHT (385)	T	T
45 F	*rohlfsi*	Poakwe, Pawnpawmnya	Ghana	1974	WRIGHT (274)	T	V
46 W	*rohlfsi*	Bobo-Dioulasso	Upper-Volta	1976	SELLIN	T	T
47	*contortus*	Goulmina	Morocco	1967	CORNU	V	T
48	*rohlfsi*	Pokoase	Ghana	1975	WRIGHT (388)	V	T
49	*rohlfsi*	Nyombe	Cameroun	1973	WRIGHT (264)	V	T
50	*rohlfsi* ?	Lukala River	Zaire	1972	RIPERT	V	T

For organ specificity studies the various organs were dissected out of live snails from one locality. This method was found superior to killing by deep-freezing and then dissecting out the organs. In order to obtain sufficient tissue for homogenisation it was found necessary to collect organs from 4 snails except in the case of blood and digestive gland, where one snail gave sufficient material. In order to obtain comparable results the wet weights of the organs were determined, and an amount of water corresponding to the wet weight was added prior to homogenisation. For the blood no water was added, and for the digestive gland an additional 1 : 5 dilution was prepared.

Results.

The results presented are based on electrophoresis of more than 400 specimens, of which 82 were wild snails (table 2) and the remaining were laboratory bred. Samples of at least 10 individuals from each of 24 stocks maintained in this laboratory revealed no variation within the stocks. Accordingly it seems reasonable to accept 2 or 3 individuals representing stocks maintained by WRIGHT (see F in table 1). In only one of the 9 wild samples was more than one phenotype observed. In pilot experiments the zymograms of both phosphoglucose isomerase and esterase were constant after one week of starvation and after changes in the diet. No effect of snail size was seen.

Organ specificity

In figure 2 the pattern obtained using whole animal extracts are shown for phosphoglucose isomerase and for esterase after 5 respectively 60 minutes staining. In phosphoglucose isomerase only one band appeared (fig. 2A), whereas in esterase a number of bands was found. Some of these bands are so heavily staining that they obscure other bands present (fig. 2B and C). For this reason it was decided to investigate the organ specificity of esterases. The result of the organ specificity studies is shown in figure 3. In albumen-, muciparous- and salivary glands esterase activity is very low. In the blood two distinct esterase

Table 2. Numbers of not laboratory bred individuals surveyed in the wild samples.

Population no.	Number of individuals.
4, 4a	12
8	14
19	9
20	11
21	10
22	4
33	10
39	2
46	10
Total	82

zones are present at a mobility of approximately twice the mobility of haemoglobin. These two esterase zones are also seen in the various organs, which is not surprising as the blood is present in all organs. In the gonad, the stomach and the digestive gland electropherograms the two heavily staining bands appear. The activity of these bands is by far the highest in the digestive gland. In the following these bands will be called Est-d or digestive gland esterases. In the snail the digestive gland and the gonad lies closely together with tissue of the two organs intersperced. The activity of Est-d in the gonad is low compared to the activity found in the digestive gland and most probably Est-d activity of the gonad is caused by some contamination of the preparation with digestive gland tissue.

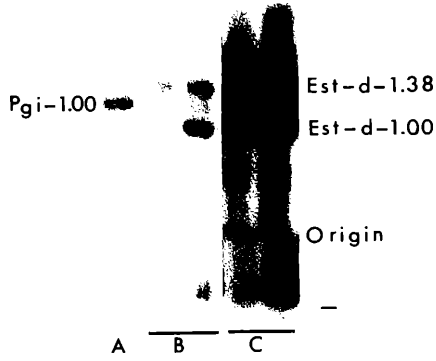

Fig. 2. Electropherograms of *Bulinus truncatus* whole animals extracts. A. Phosphoglucose isomerase. B and C. Esterases of identical samples photographed after 5 respectively 60 minutes staining.

Fig. 3. Electropherograms of esterase in various organs. A concentrated digestive gland, B kidney, C foot and buccal mass, D blood, E salivary glands, F muciparous gland, G albumen gland, H stomach, I diluted digestive gland and J gonad. The Est-d phenotype shown is Est-d-1·04/0·93.

In phosphoglucose isomerase the various organs showed nearly identical activity apart from the blood were no phosphoglucose isomerase activity was visualised.

Geographical variation in phosphoglucose isomerase.

In 48 of the samples only one band was observed, this band had the same mobility in all individuals surveyed. The mobility of this band is arbitrarily set to 1·00 and the phenotype is called Pgi-1·00. Other bands observed will be designated according to their mobility relative to this band. In samples no. 37 and 45 an identical triplebanded structure was found. This phenotype is called Pgi-1·14/1·07/1·00. The middle band is included in the type designation as this phenotype is not ascribable to normal allozyme variation, because neither the Pgi-1·14 nor the Pgi-1·00 phenotype have been observed in any individual of the two samples. This lack of segregation in the phosphoglucose isomerase phenotypes points towards parthenogenetic reproduction occuring in *Bulinus truncatus*. This mode of reproduction is commonly observed in polyploid organisms.

Geographical variation in digestive gland esterases.

As described above the digestive gland of *Bulinus truncatus* is specific for some heavily staining esterase zones. Each band observed is designated Est-d-x.yz, where the figure x.yz is calculated as the mobility of the band in question relative to the slow band of Est-d in sample no. 1. Phenotypes are indicated by the relative mobilities of the bands present e. g. Est-d-1·00/0·91.

In table 3 the Est-d phenotypes observed in the different samples are given. In all samples either the Est-d-1·00 or the Est-d-1·04 band was observed, in 45 of the samples an additional band was seen. The three most common pheno-

Table 3. Est-d phenotypes and morphological types of the samples.

Locality number	Est-d phenotype	Morphological type (MANDAHL-BARTH 1965)
1-5	1·00/1·38	eastern
4a, 7, 8	1·00/0·71	eastern
6	1·00/0·42	eastern
9-32	1·00/0·91	eastern
33	1·00	eastern
34	?	eastern
35	1·00/?	eastern
36	1·00	eastern
37-46	1·04/0·93	western
47	1·04/0·73	western
48	1·04	western
49	?	western
50	1·00/1·38	western ?

types observed are Est-d-1·00/1·38 (5 samples), Est-d-1·00/0·91 (24 samples) and Est-d-1·04/0·93 (10 samples). The remaining phenotypes have mostly been seen only in one population. The Est-d-1·00/1·38 phenotype is found in samples from Egypt and Israel, which is the area where *Bulinus truncatus truncatus* (AUDOUIN) occurs (MANDAHL-BARTH 1965). The Est-d-1·00/0·91 phenotype have been observed on Sardinia, in Yemen, Sudan and from there southwards to Malawi. In this area the subspecies *rivularis* (PHILIPPI), *sericinus* (JICKELI) and *trigonus* (MARTENS) are found (MANDAHL-BARTH 1965). The Est-d-1·04/0·93 phenotype have been observed in samples from Libya, Marocco, Portugal and West-Africa. In this area the species *contortus* (MICHAUD) and *t. rohlfsi* (CLESSIN) are distributed (MANDAHL-BARTH 1965). In no case segregation of the two Est-d bands was seen, and therefore may the two bands be controlled by two different genes, but a parthenogenetic mode of reproduction can also account for the lack of segregation observed and hence a single gene nomenclature has been applied.

Discussion.

The very great uniformity observed in the phosphoglucose isomerase pattern is surprising as this enzyme in many investigations on enzyme polymorphisms show a great deal of both inter- and intrapopulation variation (WILKINS & MATHERS 1973, JELNES 1975). In the present case this enzyme unifies, by its constancy, samples taken over the area of a continent. In the egg-proteins a similar situation has been observed as *truncatus* in these exhibits a characteristic triplebanded structure (WRIGHT & ROSS 1965, Brown & WRIGHT 1972).

The size of the first lateral tooth have by MANDAHL-BARTH (1958, 1965) been used to divide *truncatus* into an eastern and a western group. This division is strongly supported by the Est-d patterns. Even though widely separated the samples show a very great uniformity in the Est-d patterns. Samples nos. 1-36 within the eastern group area are united by the Est-d-1·00 band. In the western group area samples nos. 37-49 are characterised by the Est-d-1·04 band.

The uniformity of Est-d patterns is a strong indication of the taxonomic value of this character at the subspecific level.

The Est-d-1·04 group. Within this group no further division based on esterase pattern is justifiable. The material includes samples which would be classified as *contortus* (nos. 37-39) or as *truncatus rohlfsi* (nos. 40-50). Some evidence is provided by the esterase pattern findings for the maintenance of *contortus* as a species different from *truncatus*. A full elucidation of this problem would require a survey of several more enzymes and populations. However, in a pilot survey using more than ten enzymes it has not been possible to find clearcut electrophoretic differences between *truncatus* and *contortus*. On this evidence it seems reasonable for the time being to consider *contortus* as no more than an infraspecific form of *truncatus*.

Characters in the shell and in the size of the mesocone of the first lateral tooth are additional characters that unify the samples of the Est-d-1·04 group (MANDAHL-BARTH 1965 and personal communication). *B. truncatus rohlfsi*, originally described from lake Chad, is the name commonly applied to popu-

lations of *truncatus* in West Africa. Morphological characters seem to indicate that *truncatus* from lake Chad rather belong to the eastern group of MANDAHL-BARTH (1965 and personal communication) characterised by Est-d-1·00 in electrophoresis; should this be the case then it might be argued that *rohlfsi,* as applied in West Africa, should be replaced by *contortus.* However, the infraspecific taxa of *truncatus* are not yet fully understood and to avoid further taxonomic confusion, I therefore suggest the following provisional use of subspecific names in the Est-d-1·04 group of *truncatus*: *contortus* in Libya, North-West Africa and the Iberian Peninsula, *rohlfsi* in West Africa.

The Est-d-1·00 group. Within the area represented by this group a number of subspecies of *truncatus* have been recognised according to characters of the shell and radula (MANDAHL-BARTH 1965). These are *truncatus truncatus* from Israel, Egypt, Lebanon, Iraq, Sudan and northern Uganda, *truncatus rivularis* from Sardinia, Sicily and Corsica, *truncatus sericinus* from Ethiopia, Yemen and Aden, and *truncatus trigonus* from lake Victoria, lake Edward and lake George.

The electrophoretic patterns obtained in this investigation generally supports this subspecies division. *B. t. truncatus* is characterised by the Est-d-1·00/1·38 phenotype Uncommon phenotypes are encountered in samples nos. 4, 6, 7, and 8. Further material from this area is very desirable in order to clarify the distribution of the esterase patterns. The two subspecies *sericinus* and *trigonus* represented by samples nos. 11-32 are identical in the electrophoretic pattern by having the Est-d-1·00/0·91 phenotype. There are neither parasitological (SOUTHGATE & KNOWLES 1975, FRANDSEN 1977) nor morphological (MANDAHL-BARTH personal communication) evidence for maintaining the two subspecies. WU & BURCH (1975) compared laboratory bred populations of *truncatus truncatus* from Egypt and *truncatus sericinus* from the type locality. In spite of the title "*Bulinus sericinus* from Ethiopia" they reach the conclusion that *sericinus* is to be considered as a subspecies of *truncatus.* Based on the evidence presented here it seems as if one subspecies of *truncatus* is occuring in eastern Africa, however no material of *trigonus* has yet been available from near the type locality in the southwestern part of lake Victoria. If *truncatus* is represented by a single form in eastern Africa the senior name for it is *sericinus.*

Samples nos. 9 and 10 from Sardinia are electrophoretically indistinguishable from *truncatus sericinus.* However, since the populations on the Mediterranean islands are isolated by the great distance from the populations in Ethiopia and East Africa and a different phenotype occurs in Egypt and the Middle East it is possible, that the similarity observed is due to convergent evolution. Whether the populations on the Mediterranean islands merit recognition as a distinct taxon is an open question.

Sample no. 50. This is the only sample which have been available of *truncatus* from West Africa south of the equator. The pattern observed in this sample is identical with the pattern observed in *truncatus truncatus.* This similarity is probably incidental, but, when more material are investigated, it might turn out that *truncatus* in West Africa south of the equator are more closely related to the eastern *truncatus* group than to the western group, perhaps as a separate subspecies.

The wild samples.

Wild samples are in this context defined as composed of individuals, originating directly from the natural habitat, which have been surveyed in electrophoresis before more than 25% of the original snails have died in the aquaria. In table 2 the numbers of wild snails surveyed are given. For most of the localities additional material, classified as laboratory bred has been examined. Only one of the wild samples showed intrapopulational variation. In population 4 from Egypt the phenotypes Est-d-1·00/1·38 and Est-d-1·00/0·71 were observed respectively in 4 and 8 individuals. Neither of these two phenotypes can be interpreted as heterozygotes, and hence no possible heterozygotes have been seen. Due to chance alone this is a very unlikely situation, the probability of fit to Hardy-Weinberg proportions using Chi-square test is less that 0·001, so this observation could be taken as an indication of a parthenogenetic mode of reproduction in *Bulinus truncatus*.

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I take this opportunity to thank in anticipation those people, who in the future will send live material to the Danish Bilharziasis Laboratory. The material will be used to fill gaps in the knowledge of *Bulinus* and *Biomphalaria* taxonomy.

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References.

BROWN, D. S. (1976): A tetraploid freshwater snail (Planorbidae: *Bulinus*) in the Highlands of Kenya. — J. nat. Hist., **10**: 257-267.

BROWN, D. S. & WRIGHT, C. A. (1972): On a polyploid complex of freshwater snails (Planorbidae: *Bulinus*) in Ethiopia. — J. Zool. London, **167**: 97-132.

BURCH, J. B. & LINDSAY, G. K. (1967): Electrophoretic analysis of esterases in *Bulinus*. — Ann. Rep. am. malac. Union, **34**: 39-40.

CLAUGHER, D. (1971): Karyotype analysis of bulinid snails. — Bull. Wld. Hlth. Org., **45**: 855-858.

COLES, G. C. (1969a): Isoenzymes of snail livers. — I. Hydrolysing enzymes and peroxidase. — Comp. Biochem. Physiol., **29**: 403-411.

— — — (1969b): Isoenzymes of snail livers. — II. Dehydrogenases. — Comp. Biochem. Physiol., **31**: 1-14.

— — — (1970): Enzyme electrophoresis and speciation of *Schistosoma* intermediate hosts. — Parasitology, **61**: 19-25.

FRANDSEN, F. (1976): The suppression, by *Helisoma duryi*, of the cercarial production of *Schistosoma mansoni*- infected *Biomphalaria pfeifferi*. — Bull. Wld. Hlth. Org., **53**: 385-390.

— — — (1977): Studies of the relationship between *Schistosoma* and their intermediate hosts. I. The genus *Bulinus* and *Schistosoma haematobium* from Egypt. — J. Helminth. (in print).

JELNES, J. E. (1971): Identification of hexokinases and localisation of a fructokinase and a tetrazolium oxidase locus in *Drosophila melanogaster*. — Hereditas, **67**: 291-293.

— — — (1974): Genetics of three isoenzyme systems in *Aricia artaxerxes* F. (Lep., Rhopalocera). — Hereditas, **76**: 79-82.

— — — (1975): Isoenzyme heterogeneity in Danish populations of *Aricia agestis* (Lepidoptera, Rhopalocera). — Heretidas, **79**: 53-60.

LO, C. T., BURCH, J. B. & SCHUTTE, C. H. J. (1970): Infection of diploid *Bulinus* s. s. with *Schistosoma haematobium*. — Malac. Rev., **3**: 121-126.

MANDAHL-BARTH, G. (1958): Intermediate hosts of *Schistosoma*. African *Biomphalaria* and *Bulinus*. — Wld. Hlth. Org. Monogr. Ser., **37**: 132 S.; Geneva.

— — — (1965): The species of the genus *Bulinus*, intermediate hosts of *Schistosoma*. — Bull. Wld. Hlth. Org., **33**: 33-44.

MANDAHL-BARTH, G., FRANDSEN, F. & JELNES, J. E. (1976): *Bulinus* sp. (2n = 36) from Salisbury, Rhodesia, a close relative of *Bulinus truncatus* (AUDOUIN), being a potential intermediate host of *Schistosoma haematobium* in south-east Africa. — Trans. r. Soc. trop. Med., Hyg., **70**: 88.

SICK, K. (1965): Haemoglobin polymorphism of the cod in the Baltic and the Danish belt sea. — Hereditas, **54**: 19-48.

SOUTHGATE, V. R. & KNOWLES, R. J. (1975): Observations on *Schistosoma bovis* SONSINO, 1876. — J. nat. Hist., **9**: 273-314.

WILKINS, N. P. & MATHERS, N. F. (1973): Enzyme polymorphisms in the European oyster, *Ostrea edulis* L. — Anim. Blood Grps. biochem. Genet., **4**: 41-47.

WRIGHT, C. A. (1971): *Bulinus* on Aldabra and the subfamily Bulininae in the Indian Ocean area. — Phil. Trans. r. Soc. London, (B) **260**: 299-313.

WRIGHT, C. A. & FILE, S. K. (1968): Digestive gland esterases in the genus *Bulinus* (Mollusca, Planorbidae). — Comp. Biochem. Physiol., **27**: 871-874.

WRIGHT, C. A., FILE, S. K. & ROSS, G. C. (1966): Studies on the enzyme systems of planorbid snails. — Ann. trop. Med. Parasit., **60**: 522-525.

WRIGHT, C. A. & ROSS, G. C. (1965): Electrophoretic studies of some planorbid egg-proteins. — Bull. Wld. Hlth. Org., **32**: 709-712.

— & — (1966): Electrophoretic studies on planorbid egg-proteins. — Bull. Wld. Hlth. Org., **35**: 727-731.

WU, S. K. & BURCH, J. B. (1975): *Bulinus sericinus* (Gastropoda: Planorbidae) from Ethiopia. — Malac. Rev., **8**: 31-46.