Comp. Biochem. Physiol., 1974, *Vol.* 47**A**, *pp.* 441 *to* 446. *Pergamon Press. Printed in Great Britain*

SEROLOGICAL DIFFERENTIATION OF TWO SPECIES OF *TATERILLUS* (RODENTIA, GERBILLIDAE) FROM SENEGAL: *T. GRACILIS* (THOMAS, 1892) AND *T. PYGARGUS* (CUVIER, 1832)

J. C. BARON[1], B. HUBERT[1], P. LAMBIN[2] and J. M. FINE[2]

[1]Office de la Recherche Scientifique et Technique Outre-Mer, 24 rue Bayard, Paris (8°); and [2]Laboratoire d'Immunochimie, Centre National de Transfusion Sanguine, 6 rue Alexandre-Cabanel, Paris (15°), France

(*Received* 9 *April* 1973)

Abstract—1. The sera of two species of *Taterillus*, *T. gracilis* and *T. pygargus*, were analysed by electrophoresis with different media: acetate, starch gel and acrylamide.

2. The two principal fractions, albumin and transferrin, appear to have different mobilities according to the species and consequently can be used to differentiate these two species which are indistinguishable by the usual tests.

3. The hybrids inherit albumin and transferrin from their parents and show two different fractions for transferrin and two different fractions for albumin.

4. The chromosomal equipment of each species, 36/37 chromosomes for *T. gracilis*, 22/23 chromosomes for *T. pygargus* and 30 chromosomes for hybrids, bears out serological differentiations.

INTRODUCTION

THE TAXONOMY of the genus *Taterillus* (Thomas) has always posed problems. Recently, Rosevear (1969) regrouped all Senegalese *Taterillus* into only one species: *Taterillus gracilis* (Thomas, 1892). Matthey carried out a cytotaxonomic investigation on specimens collected in the field; there are at least two chromosomically distinct species: the first with 22 chromosomes for females and 23 for males, and the second with 36 and 37 chromosomes. Although we have never found other chromosomal numbers in nature, hybrids have been obtained with 30 chromosomes by interbreeding the two species (either by mating a male with 23 chromosomes with a female with 36 chromosomes or the reciprocal cross).

According to a recent proposal of nomenclature (Petter *et al.*, 1972), the individuals with 36/37 chromosomes are called *T. gracilis* (Thomas) and the others with 22/23 chromosomes *T. pygargus* (Cuvier). These two species, sympatric on the main part of the Senegalese territory, are indistinguishable by the usual tests (colour of the coat, body and cranial measurements and morphology, etc.) To avoid the sacrifice of the animal—prohibiting any ecological work—for chromosomal determination Hubert & Baron (1973) conducted serological investigations

by starch gel electrophoretical analysis. The present report presents the results obtained with two other different media and the localization of the transferrin.

MATERIALS AND METHODS

Samples of sera for investigation were obtained from 17 *Taterillus pygargus*, 13 *T. gracilis* and 2 hybrids. The blood was obtained by cardiac puncture. The animals studied in this work, were collected in the field (numbers with B or E) or inbred in the laboratory (numbers with Tu, see Table 1). The B-animals came from the north of Senegal, near the Pete-Ole well (16°10′ N, 15°05′ W) and the E animals were collected in the protected forest of Bandia (14°35′ N, 17°01′ W). The animals indicated as coming from the laboratory are offspring reared here, for instance the hybrids. Hybrids have never been available in the field. The two species, although sympatrics, share the ground according to their ecological requirements. Nevertheless, no sexual attraction seems to exist. On the other hand, on breeding they give birth to hybrids which are definitively sterile.

Three types of electrophoresis

Cellulose acetate electrophoresis was carried out on Cellogel bands (5·7 × 14 cm) with an apparatus manufactured by Sebia. A voltage of 200 V was applied for 45 min. The buffer was made of barbital (1·38 g)/barbital sodium (8·76 g)/calcium lactate (0·384 g) in 1 l. of distilled water, pH 8·6. The bands were stained in a red Ponceau S solution.

Horizontal starch-gel electrophoresis was carried out using a discontinuous buffer system (Smithies, 1955). The gel, 12% starch (Connaught Medical Research Laboratories, Toronto), was made up in a buffer containing 0·05 M Tris and 0·008 M citric acid, pH 8·2; the electrode vessels contained 0·025 M LiOH and 0·1 M boric acid, pH 8·4. The samples migrated in the same run with a voltage gradient of 4·5 V/cm applied for 6·5 hr at room temperature, using an apparatus already described (Baron, 1972). After electrophoresis, the gel was cut horizontally, one slice being stained with Amido black and the other revealed by autoradiography.

Electrophoretic separation according to size and charge was performed in a 4–30% concave polyacrylamide gel gradient as described by Margolis & Kenrick (1968) and using the Gradipore reagents and apparatus as supplied by the manufacturer (Townson & Mercer, Lane Cove, Australia). The buffer used was a Tris–borate EDTA buffer, pH 8·6. Five μl of each sample was submitted to electrophoresis.

Autoradiography

The serum was previously labelled with radioactive iron (^{59}Fe) by the addition of $^{59}FeCl_3$ (50 μl/ml of serum of a $^{59}FeCl_3$ solution containing 10 μCi). After starch gel electrophoresis one slice was cooled at −15°C to prevent the proteins from diffusing during autoradiography which was performed for 48 hr with a Kodirex film (13 × 18 cm) set on the gel inside a dark box kept at −15°C.

Cytological preparations of chromosomes

Caryotypes were obtained by "squashes" prepared by the method perfected by Matthey. Animals were sacrificed 1·5 hr after an intraperitoneal injection of 1% colchicine (0·01 ml/g). The spleen and gonads were collected and minced into fragments incubated for 10 min in a hypotonic solution of 1% sodium citrate, and then fixed in 50% acetic acid solution for 40 min. Each fragment was squashed on an albumenized slide with a cover slide and petroleum jelly. After immersion into 70° alcohol the preparations were coloured (acid Kemalin), dehydrated and set with Canada balsam.

The specimens were preserved, with skin and skull kept in the laboratory collection.

RESULTS

Electrophoretic analysis

Cellulose acetate (Fig. 1A). The electrophoregram shows five main protein fractions. The most important one, albumin, has the fastest relative mobility. The second important main fraction is transferrin. The transferrin has been identified in this medium by analogy with its position after paper electrophoresis followed by autoradiographic revelation.

An important mobility difference is observed between the albumin of *T. gracilis* (fast) and the albumin of *T. pygargus* (slow) (Fig. 1A). The last one does not migrate as far as the first one. The hybrid shows two albumin fractions whose mobilities correspond to those of the parents as can be seen with a pool of sera from *T. gracilis* and *T. pygargus*. The mobility of the transferrin depends also on the species: fast for *T. pygargus* and slow for *T. gracilis*, the hybrids having two transferrins transmitted from their parents.

Starch gel (Fig. 1B). Separation in this medium is better than on acetate and more than nine bands are observed on electrophoregrams. The most important and fastest fraction is albumin which shows a difference in mobility depending on the species: fast for *T. gracilis* and slow for *T. pygargus*. Hybrids have an electrophoretic pattern showing two albumin fractions, the mobilities of which are slightly different from those of the parents.

The second main fraction is transferrin localized by means of autoradiography at a fast level (No. 7) for *T. pygargus* and a slow level (No. 8) for *T. gracilis* (Fig. 1B). Another protein fraction easily visible in *T. pygargus* sera has a relative mobility close to slow transferrin and must not be confused with transferrin when using only a general Amido black 10B staining. The transferrin seems to be monomorphic in the animals of the two species studied (13 *T. gracilis* and 17 *T. pygargus*) and hybrids inherit from their parents the two different transferrins with respectively the same mobilities.

Polyacrylamide gel (Fig. 1C). This medium, with its concentration gradient (4–30 per cent), gives the best separation of *Taterillus* sera from which more than 15 fractions are well separated.

In our experiments the albumins of the two *Taterillus* species seem to migrate at the same level and, of course, the hybrid shows a one-albumin pattern. This insignificant result for albumin comes from the fact that in this kind of gel the separation mainly depends on the molecular size.

The transferrins present the same differences in mobility that are found with acetate or starch gel electrophoresis.

Caryotypes

The caryotypes studied are classified into two types according to the two species deriving from an ancestral stock. The establishment of caryotypes is complicated in Gerbillidae because of a relatively important polymorphism.

As shown in Fig. 1, the formulas established are:

T. gracilis (Thomas, 1892): 37/36 chromosomes (Fig. 1D):
Males: 37 chromosomes (fundamental number (FN) = 50):
metacentric chromosomes: 5 pairs;
acrocentric: 12 pairs;
sexual chromosomes: X/Y_1Y_2.
Females: 36 chromosomes (FN = 48):
metacentric chromosomes: 5 pairs;
acrocentric: 12 pairs;
sexual chromosomes: XX.
T. pygargus (Cuvier, 1832): 23/22 chromosomes (Fig. 1E):
Males: 23 chromosomes (FN = 46);
metacentric chromosomes: 20 pairs;
sexual chromosomes: X/Y_1Y_2.

TABLE 1—LIST OF THE *Taterillus* STUDIED WITH REFERENCE TO THE NUMBER OF CHROMOSOMES, THE MOBILITY OF THE ALBUMIN IN STARCH GEL AND IN ACETATE AND THE MOBILITY OF THE TRANSFERRIN IN STARCH GEL

No.	Sex	Chromosome number	Albumin mobility (starch gel or acetate)	Transferrin mobility in starch gel	*Taterillus* species
B 46	m	37	F	S	*gracilis*
B 74	f	22	S	F	*pygargus*
B 75	m	23	S	F	*pygargus*
B 80	f	22	S	F	*pygargus*
E 321	f	36	F	S	*gracilis*
E 324	m	37	F	S	*gracilis*
E 326	m	37	F	S	*gracilis*
E 327	m	23	S	F	*pygargus*
E 374	f	36	F	S	*gracilis*
E 375	f	22	S	F	*pygargus*
E 376	m	23	S	F	*pygargus*
E 377	f	36	F	S	*gracilis*
E 389	m	23	S	F	*pygargus*
E 390	f	36	F	S	*gracilis*
Tu 4	f	36	F	S	*gracilis*
Tu 52	f	22	S	F	*pygargus*
Tu 143	f	30	F and S	F and S	hybrid
Tu 144	m	30	F and S	F and S	hybrid
Tu 157	m	23	S	F	*pygargus*
Tu 162	f	36	F	S	*gracilis*
Tu 163	m	37	F	S	*gracilis*
Tu 175	f	22	S	F	*pygargus*
Tu 182	f	22	S	F	*pygargus*

F, Fast; S, Slow.

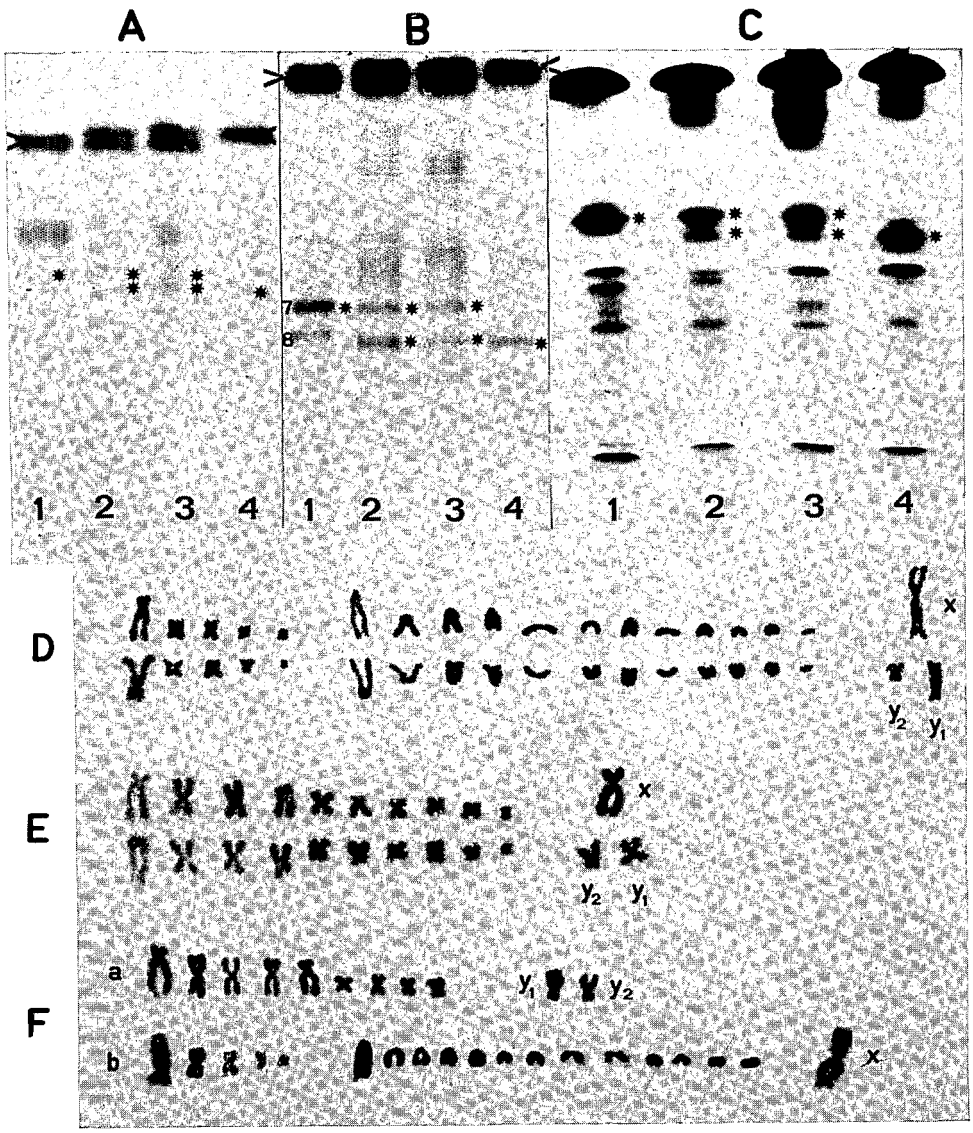

FIG. 1. A. Electrophoresis of *Taterillus* sera on cellulose acetate membrane, B in starch gel, C in polyacrylamide gradient gel. 1, *Taterillus pygargus*; 2, hybrid: *T. pygargus* × *T. gracilis*; 3, mixture of sera from one *T. pygargus* and one *T. gracilis*; 4, *Taterillus gracilis*. >, Albumins; *, zones of radioactivity (^{59}Fe) corresponding to iron-binding protein of serum (transferrin). D. Caryotype of *T. gracilis* (male, No. E 324) (×2700). E. Caryotype of *T. pygargus* (male, No. E 327) (×4000). F. Caryotype of hybrid (male, No. Tu 145) (×2700): a, chromosomes issued from male genome (*T. pygargus*); b, chromosomes issued from female genome (*T. gracilis*).

Females: 22 chromosomes (FN = 44).
Hybrids: 30 chromosomes (Fig. 1F).

The 30 chromosomes cannot be paired, but it is easy to separate what come from the male (Fig. 1F, a) and the female genome (Fig. 1F, b) For example, a male (Tu 145) is born by cross-breeding between a male *T. pygargus* and a female *T. gracilis*.

DISCUSSION AND CONCLUSIONS

Caryotypes

According to Matthey & Jotterand (1972) the Y_2 chromosome would be the half of an additional pair, the other half of which would be on the X chromosome by translocation. This kind of formula with X/Y_1Y_2 can also be found in Insectivora, Chiroptera and Artiodactyla.

The caryotypes of these two species are very similar: the fundamental numbers (FN) are not very different and some autosomes have many common characteristics (see Matthey & Jotterand, 1972). According to these authors, the 2N number and FN of these two species of *Taterillus* are less in relation to the other species of the genus, whose caryotypes are known (see Table 2).

TABLE 2—FEMALE CARYOTYPES OF SOME *Taterillus* SPECIES

	2N	FN
T. congicus	54	70
T. emini	44	68
T. gracilis from Haute Volta	36	46
T. gracilis from Senegal	36	48
T. pygargus	22	44

Electrophoretic analysis

Whereas the cytotaxonomic differentiation of *T. gracilis* and *T. pygargus* necessitates the sacrifice of animals, the serological method can be used on live animals and thus capture–marking–recapture methods are important in ecological studies. In fact only a very small amount of blood is necessary (0·1–0·2 ml) and it is quicker and easier to perform an acetate electrophoresis run (9 new serum analyses in less than 2 hr) than to prepare and examine good chromosomal preparations.

Serological work permits ecological comparative studies (qualitative and quantitative) of the two species of *Taterillus* particularly with regard to the possible different bearing of these two species in the epidemiology of some African arbovirus diseases.

Determination of the two species by the migration of the albumin on acetate is very easy and can be checked by looking at the migration of transferrin. In

order to make assurance doubly sure, starch gel electrophoresis can be done (40 samples can be analysed side by side in the same run with our apparatus) in which the various transferrins have quite different positions. An indirect method can be also used which consists of adding serum of a known species into the sample to be analysed (Hubert & Baron, 1973). If we find two albumin bands then the unknown sample belongs to the other species.

The serum electrophoretic analysis of *Taterillus* from Senegal corroborates the caryotypic analysis of Matthey & Jotterand (1972) who distinguished two cryptical species confused under the name of *T. gracilis* Thomas. If morphological and morphometrical characteristics do not permit the separation of the two species, a simple serum analysis indicates two different biochemical characters: the albumin and the transferrin.

REFERENCES

BARON J. C. (1972) Note sur un nouvel appareil d'électrophorèse horizontale pour gel d'amidon. Cah. ORSTOM, sér. *Océanogr.* X, 251–262.

BRES P., CAMICAS J. L., CORNET M., ROBIN Y. & TAUFFLIEB R. (1969) Considération sur l'épidemiologie des Arboviroses au Sénégal. *Bull. Soc. Path. exot.* **62**, 253–259.

FINE J. M. (1968) Electrophorèse en gel d'amidon. In *Techniques d'électrophorèse de zones* (Edited by DE LA TOURELLE ST.-MANDÉ).

HUBERT B., ADAM F. & POULET A. (1973) Liste préliminaire des Rongeurs du Sénégal. *Mammalia* **37**, 183–193.

HUBERT B. & BARON J. C. (1973) Determination of *Taterillus* (Rodentia, Gerbillidae) from Senegal by serum electrophoresis. *Anim. Blood Grps biochem. Genet.* **4**, 51–54.

MARGOLIS J. & KENRICK K. G. (1968) Polyacrylamide gel electrophoresis in continuous molecular sieve gradient. *Analyt. Biochem.* **25**, 347.

MATTHEY R. & JOTTERAND M. (1972) L'analyse du caryotype permet de reconnaître deux espèces cryptiques confondues sous le nom de *Taterillus gracilis* Thomas (Rongeurs, Gerbillidae). *Mammalia* **36**, 193.

MATTHEY R. & PETTER F. (1970) Etude cytogénique et taxonomique de 40 *Tatera* et *Taterillus* provenant de Haute Volta et de la République Centrafricaine (Rongeurs, Gerbillidae). *Mammalia* **34**, 585–597.

NADLER C. F., HOFFMAN R. S. & PIZZIMENTI J. J. (1971) Chromosomes and serum proteins of prairie dogs and a model of *Cynomys* evolution. *J. Mammal.* **52**, 545–555.

PETTER F. (1971) Nouvelles méthodes en systématique des Mammifères. Cytotaxonomie et élevage. *Mammalia* **35**, 331–369.

PETTER F., POULET A. R., HUBERT B. & ADAM F. (1972) Contribution à l'étude des *Taterillus* du Sénégal, *T. pygargus* (F. Cuvier, 1832) et *T. gracilis* (Thomas, 1892). (Rongeurs, Gerbillidae). *Mammalia* **36**, 210.

ROSEVEAR D. R. (1969) *The Rodents of West Africa.* British Museum, London.

SMITHIES O. (1955) Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. *Biochem. J.* **61**, 629–641.

Key Word Index—Albumin; transferrin; *Taterillus*; chromosomes; electrophoresis; cytotaxonomy.