

The House mouse complex of species: Sympatric occurrence of biochemical groups *Mus* 2 and *Mus* 4 in Rumania

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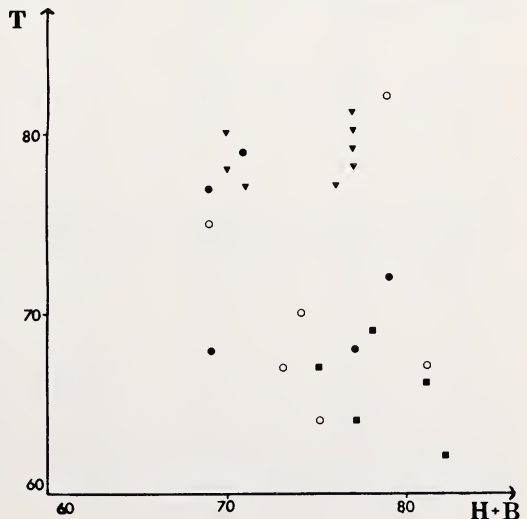
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Abstract

For the first time biochemical groups *Mus* 2 and *Mus* 4 have been shown to behave as sympatric species. *Mus* 2 is related to *Mus musculus musculus* while *Mus* 4 is related to the short-tailed mouse referred to in Greece as "*Mus musculus spicilegus*".

Introduction

Four biochemical groups (*Mus* 1 to 4) have been shown to occur among European mice on the basis of electrophoresis. *Mus* 1 and *Mus* 2 are allopatric but intergrade along a narrow hybrid zone well studied in Denmark (HUNT and SELANDER 1973). Thus they interact as parapatric semi-species of the same biological species. *Mus musculus domesticus* and *M. m. brevirostris* belong to *Mus* 1, while *M. m. musculus* and maybe other subspecies such as the Asiatic *M. m. wagneri* belong to *Mus* 2. *Mus* 3 is restricted to the Western Mediterranean where it occurs sympatrically with *Mus* 1 (BRITTON et al. 1978; THALER et al. 1978). *Mus* 1 and *Mus* 3 hybridize under laboratory conditions (BONHOMME et al. 1978a) but never do so in nature, even in such outdoor habitats where both groups are established permanently. Thus *Mus* 1 and *Mus* 3 interact as sympatric species and the latter deserves a specific name of its own (*Mus spretus*). *Mus* 4 has been defined in Greece where it occurs sympatrically with *Mus* 1, the two groups never hybridizing in nature (BONHOMME et al. 1978b). The



Tail length (T) and head and body length (H + B) distribution. This figure shows that this most used criterion is adequate in distinguishing *Mus* 1 (tail always longer than head and body) from *Mus* 4 (tail always shorter). But *Mus* 2 populations are intermedate and their distribution overlaps both *Mus* 1 and *Mus* 4 distributions. Relative tail length is not a good criterion for distinguishing the two species of Rumanian mice.

- *Mus* 2 - Bucharest; ○ *Mus* 2 - Braila;
- *Mus* 4 - Braila; ▼ *Mus* 1 - Greece

Greek shorttailed mice referred to as "*Mus musculus spicilegus*" by ONDRIAS (1965) are equivalent to the biochemical group *Mus* 4. They are easy to distinguish morphologically from mice of biochemical group *Mus* 1 which are always long-tailed and are referred to as *M. m. brevirostris* or *M. m. domesticus* by ONDRIAS depending on the color of the belly.

So it is clear that the Greek "*spicilegus*" mice (= *Mus* 4) interact with *Mus* 1 as a sympatric species. They deserve a species name of their own and we have been calling them *Mus spicilegus*. But two other questions remain unsolved:

1. What about the famous mound-builder (*tumulus*) mice which, as the Greek short-tailed mice, are also currently referred to as "*Mus musculus spicilegus*" (HAMAR 1960)?

2. What about the relationships between "*spicilegus*" (whatever its rank, species or subspecies) and *Mus musculus musculus*? According to SERAFINSKI (1965) and many others, *M. m. musculus* is an indoor mouse which has evolved in North Eastern Europe from the outdoor wild mouse *spicilegus* (including the mound-builder mice) widespread in South Eastern Europe.

Materials and methods

Eleven mice have been trapped in the great island of Braila, in a field where mound-building activity occurs each year at fall. At first sight all these mice could be referred to as "*M. m. spicilegus*". Five other mice have been trapped indoors in Bucharest city.

The mice have been analyzed by starch gel electrophoresis, using the technique described by SELANDER et al. (1971) with minor modifications.

Twenty-seven loci have been scored: 2 lactate dehydrogenases (1.1.1.27; Ldh-1 and Ldh-2); 2 malate dehydrogenases (1.1.1.37; Mor-1 and Mor-2); 2 isocitrate dehydrogenases (1.1.1.42; Id-1 and Id-2); a malic enzyme (1.1.1.40; Mod-1); an alcohol dehydrogenase (1.1.1.1.; Adh-1); a sorbitol dehydrogenase (1.1.1.14; Sdh-1); an alpha-glycerol phosphate dehydrogenase (1.1.1.8; Gdc-1); 7 esterases (3.1.1.1.; Es-1, Es-2, Es-3, Es-10, Es-14, Es-15 and Es-A); 2 glutamate oxaloacetate transaminases (2.6.1.1.; Got-1 and Got-2); a phosphoglucomutase (2.7.5.1.; Pgm-2); a glucosephosphate isomerase (5.3.1.9.; Gpi-1); an indophenol oxidase (1.15.1.1.; Ipo-1); a carbonic anhydrase (4.2.1.1.; Car-2); 2 non-enzymatic proteins (hemoglobin β chain Hbb and albumine Alb) and a gene regulating lactate dehydrogenase subunit B activity in red cells (Ldr-1). For the purpose of comparison populations of *Mus* 2 from Denmark from a locality close to Aarhus, and of *Mus* 1 and *Mus* 4 from Greece (near Thessaloniki) have been analyzed at the same loci.

From the gene frequencies various indexes have been computed (Table 2):

intrapopulation genic heterogeneity: $H = \sum \frac{1 - \sum x_i^2}{n}$

NEI's genetic distance (1972): $D = - \text{Ln} I = \frac{1}{n} \sum_{j=1}^{j=n} \frac{\sum_i x_i y_i}{\sqrt{\sum x_i^2 y_i^2}}$

and the index: $A = n - \sum_{j=1}^{j=n} (\sum_i x_i y_i)$ (AUTEM et BONHOMME 1980)

in which x_i and y_i are the frequencies of the i^{th} allele at the j^{th} locus in populations X and Y.

Length of tail and body have been measured. Hybridization experiments have been attempted.

Results

The mice analyzed in this study were found to belong to two distinct biochemical groups on the basis of electrophoretic data. Each group shows different alleles at 6 loci (Table 1) while identically homozygous at the 10 following loci: Ldh-1, Ldh-2, Mor-1, Mor-2, Gdc-1, Got-1, Es-14, Id-2, Gpi-1, and Es-A.

A cross has been obtained between a male *Mus* 4 from Rumania and a female *Mus musculus* (C 57 Bl/6 inbred stock). Among a litter of five, three were expelled dead and two vigorous female hybrids grew to unusual large size and may be sterile.

From the gene frequencies various indexes have been computed (Table 2).

Table 1

Allelic frequencies at the 17 loci that are not identically monomorphic in populations of *Mus* 1, *Mus* 2 and *Mus* 4

Locus	Allele	<i>Mus</i> 2			<i>Mus</i> 1	<i>Mus</i> 4	
		Bucharest	Braila	Denmark	Greece	Braila	Greece
Id-1	100	0,00	0,00	0,00	0,60	1,00	1,00
	125	1,00	1,00	1,00	0,40	0,00	0,00
Mod-1	r	0,25	0,59	0,40	0,00	0,20	0,00
	m	0,75	0,41	0,60	1,00	0,80	1,00
Hbb	s	0,67	0,14	0,50	0,85	0,00	0,00
	d	0,33	0,86	0,50	0,15	1,00	1,00
Alb-1	100	1,00	1,00	1,00	1,00	1,00	0,00
	98	0,00	0,00	0,00	0,00	0,00	1,00
Adh-1	100	0,33	0,55	1,00	1,00	0,00	0,00
	35	0,67	0,45	0,00	0,00	1,00	1,00
Sdh-1	110	0,00	0,00	0,00	0,50	0,00	0,00
	100	1,00	1,00	1,00	0,50	1,00	1,00
Got-2	f	0,67	1,00	1,00	0,80	1,00	1,00
	s	0,33	0,00	0,00	0,20	0,00	0,00
Ldr-1	a	1,00	1,00	0,00	0,85	0,70	0,70
	b	0,00	0,00	1,00	0,15	0,30	0,30
Es-1	100	1,00	1,00	1,00	0,00	0,00	0,00
	94	0,00	0,00	0,00	0,92	1,00	1,00
	92	0,00	0,00	0,00	0,08	0,00	0,00
Es-2	107	0,00	0,00	0,00	0,00	0,90	1,00
	100	0,00	0,05	0,17	1,00	0,10	0,00
	98	1,00	0,95	0,83	0,00	0,00	0,00
Es-3	105	0,00	0,00	0,00	0,00	0,50	0,00
	102	1,00	0,95	0,55	1,00	0,00	0,00
	100	0,00	0,00	0,00	0,00	0,50	0,04
	80	0,00	0,05	0,45	0,00	0,00	0,00
	70	0,00	0,00	0,00	0,00	0,00	0,96
Es-10	100	0,00	0,09	0,00	1,00	1,00	1,00
	60	1,00	0,91	1,00	0,00	0,00	0,00
Es-15	115	0,00	0,00	0,00	0,00	0,50	0,00
	110	0,00	0,00	0,00	0,00	0,50	0,00
	100	1,00	1,00	1,00	1,00	0,00	1,00
Pgm-2	r	0,92	0,75	0,55	0,92	1,00	1,00
	l	0,08	0,25	0,45	0,08	0,00	0,00
Pgd-1	100	1,00	1,00	0,85	1,00	1,00	1,00
	l	0,00	0,00	0,15	0,00	0,00	0,00
Ipo-1	100	0,00	0,00	0,00	0,92	1,00	1,00
	80	1,00	1,00	1,00	0,08	0,00	0,00
Car-2	100	1,00	1,00	1,00	1,00	0,00	0,00
	80	0,00	0,00	0,00	0,00	1,00	1,00

Table 2

Genic diversity between 3 biochemical groups using the following indexes:

H = intrapopulation genic heterogeneity; I = genetic similarity and D: genetic distance (NEI 1972); A = mean number of locus for which two randomly chosen genomes differ in the two species compared; n = 27 loci

D_{Nei}	$A_{xx} = nH_x$	A_{xy}
I_{Nei}	H_x	

		Bucharest	<i>Mus 2</i> Braila	Denmark	<i>Mus 1</i> Greece	<i>Mus 4</i> Braila	<i>Mus 4</i> Greece
<i>Mus 2</i>	Bucharest	1,86 0,079	2,51	4,185	6,83	10,07	10,96
	Braila	0,025 0,976	1,94 0,072	3,86	7,26	9,80	10,83
	Denmark	0,084 0,920	0,068 0,934	2,51 0,093	7,64	11,23	12,20
<i>Mus 1</i>	Greece	0,212 0,809	0,232 0,793	0,240 0,787	2,27 0,084	7,86	8,75
<i>Mus 4</i>	Braila	0,394 0,675	0,377 0,686	0,452 0,636	0,263 0,768	1,91 0,071	3,21
	Greece	0,476 0,622	0,466 0,628	0,543 0,581	0,338 0,713	0,080 0,923	0,513 0,019

Discussion

Mus 4 and *Mus 2* have been trapped together in a field near Braila. In this place *Mus 4* and *Mus 2* have fixed or almost fixed different alleles at the following loci: Es-2, Es-3, Es-15, Id-1, Ipo-1 and Car-2. This indicates complete genetic isolation. Thus *Mus 4* and *Mus 2* interact as sympatric species.

There is a significant genetic distance between Rumanian and Greek *Mus 4*. They exhibit different alleles at three loci: Alb-1, Es-3 and Es-15 (see Table 1 for details of gene frequency). On this basis we may define two biochemical subgroups: a northern subgroup *Mus 4 B* defined in Rumania and a Southern subgroup *Mus 4 A* defined in Greece. It remains to be known if *Mus 4 B* and *Mus 4 A* intergrade or not. Provisionally we will consider them as conspecific.

There is not much genetical divergence between the field population (Braila) and the

city population (Bucharest) of *Mus* 2 in Rumania. But the field population exhibits slightly shorter tail length than the city population and thus comes morphologically closer to *Mus* 4 B. There is a significant genetic distance between both Rumanian *Mus* 2 populations and the Danish *Mus* 2 population but this divergence is due mostly to allelic frequency variation at many loci and does not allow for a definition of biochemical subgroups in *Mus* 2.

Conclusion

This is the first time the biochemical group *Mus* 2 has been shown to occur sympatrically with another group, *Mus* 4. *Mus* 2 is found outdoors as well as indoors, whereas *Mus* 4 is found only outdoors. Outdoor specimens of *Mus* 2 are very difficult to distinguish from specimens of *Mus* 4 trapped in the same area (where mound-building activity occurs). *Mus* 2 is related to *Mus musculus musculus*, while *Mus* 4 is related to the short-tailed mouse of Greece known there as "*Mus musculus spicilegus*" (ONDRIAS 1965). The question arises as to which species, *Mus* 2 or *Mus* 4, is responsible of the mound-building activity in Rumania.

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Zusammenfassung

Der Artenkreis der Hausmaus: Sympatrie der biochemischen Gruppen Mus 2 und Mus 4 in Rumänien

Zum erstenmal wird gezeigt, daß die Mäuse, die den biochemischen Gruppen *Mus* 2 und *Mus* 4 angehören, in Rumänien sympatrisch vorkommen. *Mus* 2 gehört zu *Mus musculus musculus*; *Mus* 4 zeigt Verbindungen zu der Kurzschwanzmaus, die in Griechenland als *Mus musculus spicilegus* bezeichnet wird.

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