Chromosomal Findings in the Spiny Mice of Thailand (Genus *Mus*) and Occurrence of a Complex Intraspecific Variation in *M. shortridgei*[1]

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I. Introduction

The spiny mice of Thailand, *Mus shortridgei* and *Mus pahari*, possess 46 to 49 chromosomes in wild populations sampled by us. Such karyotypes differ so radically from those with 40 telocentric chromosomes invariably found in the native ochraceous-bellied house mouse *(Mus musculus castaneus)* and its allies in Thailand *(Mus cookii, Mus caroli* and *Mus cervicolor)* that it seems desirable to document the diploid karyology of *Mus shortridgei* and *Mus pahari* as well as the occurrence of intraspecific chromosomal polymorphism in one of these species (work of A. Gropp and A. Markvong) and to examine the relationship that *Mus shortridgei* and *M. pahari* bear to *M. musculus* (taxonomic study of J. Marshall).

II. Method

Chromosome spreads have been prepared from bone marrow cells taken from the femur of the animals which had been injected with 0,5 % colchicine solution in the amount of 0,003 ml/g body weight 1 to 3 hours preceding sacrifice. Hypotonic treatment (1,0 % aqueous sodium citrate) of the cells for 15 minutes, including time spent in expelling the cells from the femur with a stream of hypotonic solution and the five minutes of centrifugation, was followed by fixation in freshly made 3 parts methanol with 1 part glacial acetic acid, and by 2 to 3 repeated centrifugings and resuspensions in additional fixative. Spreading on dry slides and air-drying as well as staining in orcein or Giemsa solution were done according to usual techniques. In conducting the research described in this report, the investigators adhered to the „Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences — National Research Council.

III. Survey of the genus Mus and taxonomy of the mice whose karyology is discussed

All the mice discussed or mentioned herein share these unique attributes of the genus *Mus:* the length of the first upper molar is more than half the toothrow, there is no postero-internal cusp on this tooth, the plantar pad is small and round, and there are peculiar fenestrations in the side of the cranium, at least in the young. The latter include a prelambdoidal fenestra flanked by a slender paraoccipital process. The anterolateral corner of the parietal bone of the genus *Mus* projects forward in a point.

[1] Dedicated to Professor Sajiro Makino, University of Sapporo, Japan.

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Table 1

	Field no.	Captured, locality	Date Sex	Number of chromosomes: acrocentric	subtelocentric	metacentric: medium (size)	metacentric: small (size)	Total	F.-N. (fundamental no.)	Museum where preserved
1	196	Mae Klang Chomtong, Chiangmai Prov.	June 1970 ♂	44		2		46	48	ASRCT
2	6872	Sakaerat, Paktongchai, Khorat Province	Sept. 1971 ♂	44		2		46	48	MAK
3	6861	„	June 1971 ♂	44	1	1		46	48	MAK
4	6908	„	Sept. 1971 ♀	44	1	1		46	48	MAK
5	155	„	Sept. 1969 ♀♂	44		2	1	47	50	ASRCT
6	163	„	Sept. 1969 ♀♂	44		2	1	47	50	ASRCT
7	6862	„	Sept. 1971 ♀♂	44		2	1	47	50	MAK
8	170	„	Sept. 1969 ♀♀	44	1	1	2	48	52	ASRCT
9	6905	„	Sept. 1971 ♂	44	2	1	1	48	52	AMNH
10	6885	„	Sept. 1971 ♀♂	45	1	1	1	48	51	AMNH
11	6884	„	Sept. 1971 ♀	46	1	1	1	49	52	MAK

AMNH = Am. Mus. Nat. Hist., New York; ASRCT = Appl. Science Res. Corp. Thailand, Centre f. Thai. Nat. Ref. Coll. Bangkhen; MAK = Mus. Alex. Koenig, Bonn.

Table 2

Field no.	Date captured	Sex	Number of chromosomes: acrocentric	Number of chromosomes: total	Museum where preserved
1 201	June 1970	♂	48	46	ASRCT
2 264	Oct. 1971	♀	48	48	MAK
3 232	litter of no 264, born Nov. 1971	♂♀	48	48	MNHN
4 233		♀	48	48	MAK
5 262		♂	48	48	MVZ
6 263		♂	48	48	MAK
7 6891	Nov. 1971	♂	48	48	BMNH
8 6906	Oct. 1971	♂	48	48	AMNH
9 —	F_1 of 264	♂	48	48	—
10 —	F_3 of 264	♂	48	48	—

BMNH = British Museum (Natural History); MNHN = Muséum National d'Histoire Naturelle, Paris; MVZ = Museum of Vertebrate Zoology, Berkeley.

There are 4 groups of species, herein called subgenera, in the genus *Mus*. First (1) is the subgenus *Leggadilla* to which *Mus shortridgei* belongs, along with the Indian species *M. saxicola, M. platythrix* and *M. phillipsi*. These mice are mostly spiny and they are rat-like in possessing supraorbital ridges for the attachment of the masticatory muscles. Second (2) is the shrewlike subgenus *Coelomys* with small eyes, long nose, and broad interorbital region. Species belonging to it are *M. pahari, Mus mayori, M. famulus, M. crociduroides,* and *M. vulcani*. The third (3) subgenus is *Mus,* usually with 40 telocentric chromosomes, without skull ridges or broad frontal bones, with long incisive foramina, and capable of living with man, either in the house or in the ricefield. Species in the subgenus *Mus* are *Mus cookii, M. nagarum, M. fulvidiventris, M. booduga, M. caroli, M. cervicolor,* and *M. musculus*. In Africa is a fourth (4) subgenus of pigmy mice *("Leggada")* with numerous species, whose chromosomes have been studied *in extenso* by MATTHEY (1963–1970) and JOTTERAND (1972).

Mus musculus is specialized in its capacity for commensalism, its short nose and diminutive teeth. *Mus shortridgei* and *Mus pahari* are about equally removed structurally and behaviorally from *M. musculus*. The degree of interrelationship of these three species could be represented as the three apices of an equilateral triangle.

IV. Results

1. *Mus (Leggadilla) shortridgei*

As shown in table 1, eleven individuals of *M. shortridgei* representing two different populations from decidous dipterocarp forest have been examined. One animal is from near the Mae Klang waterfall, Chomtong District, Chiangmai Province, the others were trapped at Sakaerat Research Station, Paktongchai District, Korat Province, Thailand. The diploid chromosome number varied among the individuals, ranging from 2n = 46 to 2n = 49.

2n = 46: Four of the eleven individuals had 46 chromosomes, all with a series of 44 acrocentrics of decreasing size and including a larger X and a very small Y-chromosome. The two other chromosomes were either represented by a presumably homozygous pair of medium sized metacentrics (Table 1, Fig. 1 and 2c) or by only

Fig. 1. Male *M. shortridgei* (no. 2, Table 1). Karyotype with a diploid number of 46 including a pair of medium sized metacentrics

Fig. 2 a—d. M. shortridgei. Selected chromosomes of karyotypes of specimens with 2 n = 46 (a = no. 2; b = no. 3; Table 1) and with 2 n = 47 (c = no. 5; d = no. 6; Table 1). Represented are the two largest autosomes, the subtelocentric chromosome, the medium and small sized metacentric

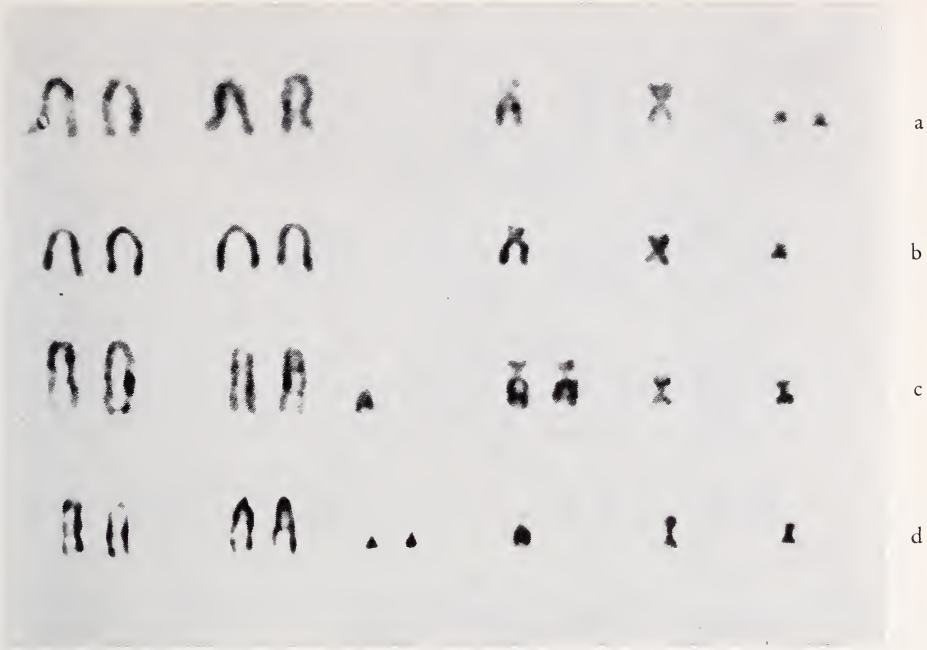

Fig. 3 a—d. M. shortridgei. Selected chromosomes of karyotypes with 2 n = 48 (a = no. 8; b = no. 10; c = no. 9; Table 1) and 2 n = 49 (d = no. 11; Table 1). The same types of chromosomes as in *Fig. 2* are respresented

one metacentric of this size and a slightly larger subtelocentric (Fig. 2a and b). Irrespective of that difference, the F. N. (= Fundamental number of chromosome arms) was 48 in all four cases.

2n = 47: The karyotype of all three animals (2 males and 1 female) with 47 chromosomes resembled closely that of the animals with 46 chromosomes and paired medium-sized metacentrics, except for the presence of an additional minute metacentric (Table 1, Fig. 2d and e). The F. N. of these animals was therefore 48 plus the two arms of the minute extra chromosome = 50.

2n = 48: This group of three animals (among 11) is heterogenous (Table 1, Fig. 3a—c). Two animals had 44 acrocentrics, one other had 45 acrocentrics. Probably, the additional acrocentric is one of the smallest of that series (Fig. 3c). Besides that, the three karyotypes vary mainly in respect to the presence and the zygosity of the subtelocentric and of the two different medium sized and minute metacentric chromosomes. The F. N. attained in this group is 48 to 50 plus the arms of minute extra-chromosomes = 51 to 52.

2n = 49: The karyotype of the single animal with the diploid chromosome number of 49 resembled closely to that of specimen no. 10 (2n = 48), except that there was one more acrocentric, most probably a very small one (Fig. 3d).

2. *Mus (Coelomys) pahari gairdneri*

The 10 specimens examined (Table 2) represent members of a population at the edge of evergreen rain forest on the shoulder of Doi Inthanon, Chomtong District, Chiangmai Province. They uniformly possess a karyotype of 48 acrocentric chromosomes

Fig. 4a. Male *M. pahari* (no. 9; Table 2) karyotype, 2 n = 48

(Fig. 4a). The X chromosome is supposedly one of the large elements. The Y chromosome belongs to the smaller acrocentrics but is possibly not the smallest. Evidence for this assumption is provided by the size of the Y chromosome in the end-to-end associated X-Y-bivalent in meiosis I (Fig. 4b).

V. Karyotypes of other species in the subgenera *Leggadilla* and *Coelomys*

a. *Leggadilla.* The karyotype of *Mus saxicola gurkha* has been given preliminary notice by Pathak (1970) under the name *Mus platythrix.* There are 22 chromosomes, most of them acrocentric including the Y, which is not however the smallest acrocentric. The largest autosomal pair is metacentric. The next largest, which is much smaller than the first, is a subtelocentric pair. The X chromosome is a subtelocentric of medium size. Pathak (1970) found the same karyotype in the two males and one female studied from Nahan, that were amongst a collection of mice trapped by the Arbovirus Research Centre, Poona, India, numbered SM 1527, SM 1528, SM 1592 and SM 1593. In response to enquiries (Marshall) concerning the identity of these mice whose karyotype contrasts so much with *Mus shortridgei,* Dr. V. Dhanda of the Poona laboratory kindly sent us two specimens collected in the same region and time as the above, which he stated to have the same morphological characteristics (Arbovirus Research Centre specimens numbers SM 1521 from Simla and SM 1588 from Nahan). They belong to the soft-furred subspecies *(gurkha)* of the species characterized by four pairs of anterior mammae and large anterior accessory cusp on the first upper molar, that should be designated as *Mus saxicola.*

Karyotypes of true *Mus platythrix* (with three anterior pairs of mammae and no accessory cusp) and spiny *M. saxicola saxicola* are being reported by Dr. Dhanda and his colleagues in a forthcoming publication. Their discovery that two species of *Leggadilla* occurred in the vicinity of Poona resulted from noting that each was parasitized by a different species of the louse, *Hoplopleura.* Sympatry at Poona is the first concrete evidence that the currently used

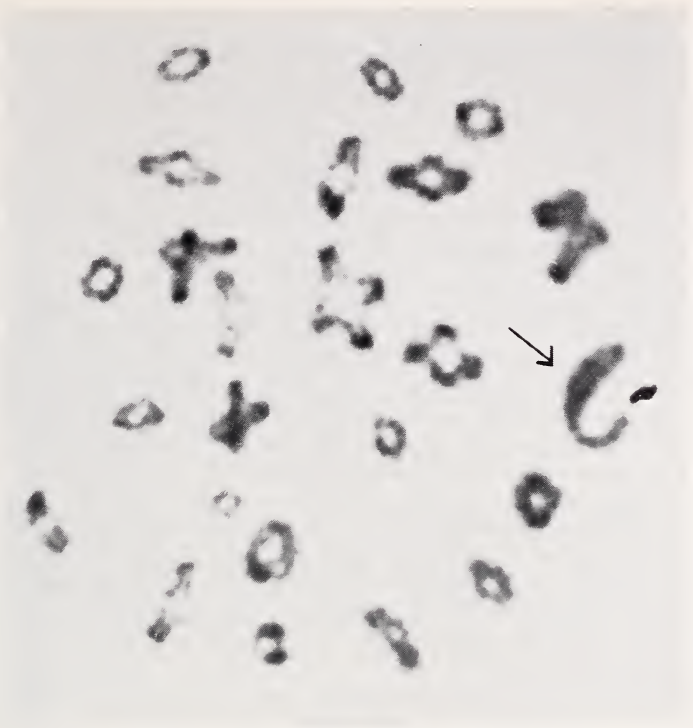

Fig. 4b. Male *M. pahari.* (no. 9; Table 2) Late diakinesis of spermatocyte I. → XY-bivalent

taxonomy of Ellerman (1947) is incorrect in assigning all forms of *Leggadilla* to the single species *M. platythrix.*

b. *Coelomys.* As to karyotypes of other species in the subgenus *Coelomys,* luck has been against our efforts to bring the mice alive down the mountains of Java *(M. vulcani).* The related *Mus famulus* of the Nilgiri Hills, Southern India, should be a prime target for chromosome study because of some peculiar skull characteristics suggestive of the subgenus *Mus.*

VI. Conclusions

While in the genus *Mus* the chromosomal constitution has been intensively studied in many species and local forms of the two subgenera *Mus* and "*Leggada*" (African pigmy mice), it is the objective of this report to present the cytogenetic findings in members of the two Asiatic subgenera *Leggadilla* and *Coelomys.* So far, the only notice on the karyotype of a species belonging to one of these groups is that of Pathak (1970) who described an odd karyotype of 2n = 22 in a small sample of animals identified by him as *M. plathythrix,* but apparently representing *M. saxicola gurkha.*

Undoubtedly, the karyotype patterns found in *M. pahari* (2n = 48; N. F. = 48) and in *M. shortridgei* (2n = 46–49; N. F. = 48–52) are different from those known in the subgenus *Mus* and in the African pigmy mice. A chromosome complement of 40 acrocentrics is common to most species or local forms of the subgenus *Mus* so far studied *(M. cooki, M. cervicolor, M. caroli, M. musculus).* Only in some predominantly alpine Swiss and Italian areas, lower diploid chromosome numbers resulting from Robertsonian changes have been found to occur in feral populations of *M.*

musculus (Gropp et al. 1970, 1972). There is, however, a remarkable constancy of the F. N. = 40 in the entire subgenus. Similarly, the F. N. is constantly 36 in the numerous local forms of the African pigmy mice, though their diploid chromosome number may vary within a „Robertsonian fan" between 2n = 18 and 34 (Matthey 1970). The karyotypes of the three Asiatic species of *Leggadilla* and *Coelomys* known at present (Pathak 1970; this report) differ strikingly from those patterns.

While the karyotype was shown to be invariably 2n = 48 in the sample of *M. pahari* described in the present report, a surprisingly extensive interindividual chromosomal variation has been shown to characterize the specimens of *M. shortridgei*. Although ten animals were captured in the same area, albeit at different time intervals, they displayed a variation of the chromosome number (2n = 46–49) together with additional structural heterozygosity of certain chromosomes. Thus, altogether seven different karyotypes could be observed in this small group of specimens of *M. shortridgei*. The basic complement (Table 1, no. 1 to 4) seems to be 44 acrocentrics and 2 medium sized subtelocentric or metacentric chromosomes that are either doubly heterozygous or homozygous for one type. This pattern is modified by the addition of one or two minute metacentrics (Table 1, no. 5 to 8) in animals with 47 and 48 chromosomes. Besides that, the findings in animals with 48 and 49 chromosomes (no. 9 to 11; Table 1) provide evidence for a tendency of each of the medium-sized subtelocentric or metacentrics to be homozygous, and also for the occurrence of a further extra chromosome belonging to the series of acrocentrics, most probably a very small one. These observations lend support to the assumption of a complex type of intraspecific chromosome polymorphism involving pericentric inversion and supernumerary chromosomes.

Both mechanisms of karyotype variation are well known in rodents, though less frequent than Robertsonian centric fusion. Thus, pericentric inversion has been shown to occur in pocket gophers (Berry and Baker 1971) in *Permoyscus* (Hsu and Arrighi 1968; Hsu and Mead 1969, Te and Dawson 1971), in mole rats (Soldatovic et al. 1967; Wahrman et al. 1969), but not in *Mus*, except one single observation of Matthey (1964) in *Mus (Leggada) minutoides*. Extra (supernumerary) chromosomes, so far reported only in few instances in mammals, have been described in Asiatic subspecies of *Rattus rattus* (Gropp et al. 1970; Pathak 1971; Yong and Dhaliwal 1972; Wahrman and Gourevitz 1972), but not yet in the genus *Mus*. *M. shortridgei* can therefore be considered as a model species displaying a combination of different types of chromosomal changes otherwise not known, or at least not represented to this extent in the genus *Mus*. It is to be expected that with the sudy of more specimens from other geographic areas, additional variations may be found. Thus, with the special karyotypes present in the samples studied in this report (Table 1), the existence of animals with diploid chromosome numbers of 2n = 50 to 52 can be predicted.

The findings in *M. shortridgei* together with the observation of Pathak (1970) of a karyotype with a diploid chromosome number of 22 including a pair of large metacentrics in *M. saxicola gurkha* are in favour of the assumption that the subgenus *Leggadilla* of *Mus* displays a high degree of chromosome variation and of evolutionary karyotype diversification. The striking chromosome differences between *M. saxicola* and *M. shortridgei* underline the necessity of clarifying the equivocal taxonomy and the hitherto obscure relationships in this subgenus.

Summary

The karyotypes of *M. shortridgei* and *M. pahari* belonging to the subgenus *Leggadilla* and *Coelomys* respectively, differ radically from those of most members of the genus *Mus*. A complex and mixed type of intraspecific polymorphism is responsible for a numerical and

structural variation of the chromosomal complement of *M. shortridgei* (2 n = 46—49; N. F. = 48—52), but the occurrence of supernumerary autosomes seems to be the most prominent feature besides pericentric inversion. — On the contrary, *M. pahari*, as revealed by the study of a limited number of specimens belonging to one colony, was shown to possess a stable karyotype with 48 acrocentric chromosomes.

Zusammenfassung

Chromosomenbefunde an Stachelmäusen aus Thailand (Genus Mus) und Beobachtungen eines intraspezifischen Polymorphismus bei M. shortridgei

Aus den Subgenera *Leggadilla* und *Coelomys* wurden *M. pahari* und *M. shortridgei* cytogenetisch untersucht. Ihr Karyotyp weicht von den meisten übrigen Arten des Genus *Mus* deutlich ab. *M. pahari* besitzt 48 acrocentrische Chromosomen. *M. shortridgei* weist einen komplexen intraspezifischen Polymorphismus mit interindividuellen Variationen der Chromosomenzahl von 46 bis 49 (N. F. = 48—52) auf. Dabei spielen pericentrische Inversionen und das Vorkommen überzähliger (Extra-, B-)Autosomen eine Rolle.

Acknowledgements

We thank Mr. Vandee Nongngork for trapping most of the mice, dissecting out the femora, and preparing the voucher specimens for museum preservation. Dr. Aroon Santadusit, Professor of Anatomy, kindly provided laboratory facilities at Chiangmai. Mrs. Margarete Bückig prepared the majority of the photomicrographs. We thank the curators of the Arbovirus Research Centre, American Museum of Natural History, and Field Museum for loaning specimens which clarified the relationships of species in the subgenera treated here. The contribution of A. G. has been made possible by a travel grant of the Deutsche Forschungsgemeinschaft (77/415/70).

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Cytogenetische Analyse der Farbvererbung bei der Tabakmaus (*Mus poschiavinus* Fatio, 1869)[1]

Von Andreas Radbruch

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Leiter: Prof. Dr. A. Gropp

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Die Tabakmaus (*Mus poschiavinus* Fatio, 1869) ist bisher nur für das Puschlavtal nachgewiesen (Fatio 1869; V. Lehmann 1968; Gropp und Mitarb. 1970). Sie ist wesentlich dunkler gefärbt als andere Hausmäuse. Sie ist durch einen insofern abweichenden Karyotyp ausgezeichnet, als sie gegenüber einem Chromosomensatz mit 20 Paaren akrozentrischer Chromosomen, wie er sich bei der Haus- und Laboratoriumsmaus findet, 6 Paar akrozentrischer und 7 Paar metazentrischer Chromosomen besitzt. Allerdings ändert sich die Grundzahl der Chromosomenarme N.F. = 40 (Nombre fondamental; Matthey 1949) nicht. Das deutet darauf hin, daß die metazentrischen Chromosomen der Tabakmaus durch eine Robertsonsche Chromosomenvariation entstanden sind (Gropp u. Mitarb. 1970). Durch Chromosomenuntersuchungen an rückgezüchteten Hybriden wurde versucht, die Frage zu beantworten, ob das für die Fellverdunkelung verantwortliche Gen auf einem metazentrischen oder einem akrozentrischen Chromosom lokalisiert ist.

Tabakmäuse wurden von Prof. v. Lehmann u. a. mit Hausmäusen der Färbung „black and tan" (*Mus musculus domesticus* Rutty, 1772) verpaart und die F1-Hybri-

[1] Herrn Prof. Dr. E. von Lehmann zum 60. Geburtstag in Dankbarkeit zugeeignet.

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