protein and enzyme determinations. 0.1 ml of a stock adenosine solution (50 µmoles adenosine/ml H_2O), 0.15 ml of a 0.1 *M* potassium phosphate buffer pH 7.4, and 0.1 ml of H_2O were incubated for 30 min at 37 °C with 0.3 ml of the supernate. Ammonia released from adenosine by deamination was determined by the method of SELIGSON and SELIGSON[8]. Protein concentrations in the supernates ranged from 12.5 to 18.9 mg/ml[9].

Results and discussion. As shown in the Table, easily measurable levels of ADA activity were found in each individual liver extract. No significant differences in this enzyme activity were found between genotypes of animals of ages less than 2 months. These findings indicate that the immunological impairment in nude mice is not associated with ADA deficiency and is not a model for this form of human combined immunological deficiency disease. Gross examination of the liver samples showed no noticeable abnormalities. Microscopy performed on the livers taken from nudes of the same age raised under identical conditions did not show necrotic foci with granulocytic infiltration that have been observed in livers of older animals. It is noteworthy that PANTELOURIS and MACMENAMIN[10] have reported an increase in L-tryosine and L-glutamine acid decarboxylase activity in the livers of nude mice greater than 2 months of age[10]. However, adult nude mice have a high incidence of liver disease and the reported enzyme differences in older mice may be due to hepatic pathology rather than related directly to the mutation[7]. In any case, mice with a selective deficiency in cellular and humoral immunity have ADA activity that is similar to their immunologically intact littermates.

Résumé. Une forme de maladie humaine d'immuno-insuffisance grave combinée est associée à une insuffisance d'adénosine-déaminase dans les cellules de lymphe et dans d'autres tissus comprenant le foie, la rate et les érythrocytes des cultures de fibroblastes de la peau. Un modèle animal d'immuno-insuffisance combinée a été récememt créé dans une race de souris sans poils. Nous avons montré que chez ces souris immunologiquement déficientes l'activité de l'adénosine-déaminase est normale.

S. I. DEUTSCH, T. J. ROMANO, N. K. GHOSH and R. P. COX[11]

Division of Human Genetics and Departments of Pharmacology, Pathology, and Medicine; New York University School of Medicine, 550 First Avenue, New York (New York 10016, USA), 5 June 1974.

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Inter-Specific Relationships of *Leiopelma* (Amphibia: Anura): Further Karyological Evidence

The genus *Leiopelma*, because of its long period of geographical isolation and its retention of a relatively large number of primitive anatomical and morphological characteristics, is of general biological interest and evolutionary importance. Among the many questions surrounding the genus are those concerning its degree of affinity with the North American amphicoelous frogs of the genus *Ascaphus* Stejneger and the inter-specific relationships within the genus *Leiopelma* itself. The present karyological investigation was undertaken in the hope that it would assist in taxonomic clarification.

Arm ratios, centromere indices and relative lengths of chromosomes of *L. hamiltoni*

Pair No.	Arm ratios[a]	Centromere indices[a]	Relative lengths[a]
1	1.18 ± 0.05	45.86 ± 1.14	1.00
2	2.12 ± 0.18	31.74 ± 0.37	0.87 ± 0.02
3	1.13 ± 0.02	46.86 ± 0.58	0.89 ± 0.02
4	1.16 ± 0.03	46.41 ± 0.53	0.81 ± 0.02
5	2.81 ± 0.08	26.37 ± 0.54	0.69 ± 0.02
6	1.22 ± 0.04	45.00 ± 0.75	0.50 ± 0.01
7	1.19 ± 0.02	45.72 ± 0.41	0.43 ± 0.01
8	1.03 ± 0.02	49.20 ± 0.34	0.39 ± 0.01
9			0.29 ± 0.01

[a] Mean ± standard error. Figures are based on measurements from 10 metaphase spreads. Lengths have been calculated in relation to the length of the longest pair. The chromosomes are shown in the same order as in Figure 2.

MORESCALCHI[1] first described the chromosomes of *Leiopelma hochstetteri* Fitzinger, the most widely distributed species of the sole endemic New Zealand genus of frogs, from 2 female specimens. Both of these had 5 pairs of metacentric or submetacentric chromosomes and 6 smaller pairs of acrocentrics but, in addition to this basic complement of 22, the karyotype of 1 specimen included 12 microchromosomes while the other had only one. Later, the present authors[2] demonstrated that the microchromosomes of *L. hochstetteri* appear to function as supernumeraries and that they show considerable individual variation in number and may even be absent.

The karyotype of a second species, *L. archeyi* Turbott, was concurrently described[2] from 2 female specimens. The total chromosome number was 18 and only the smallest pair was acrocentric. No microchromosomes were present.

Until now, the karyotype of the third known species, *L. hamiltoni* MCCULLOCH, has not been described. The occurrence of *L. hamiltoni* was originally reported from Stephens Island in Cook Strait[3] where the frog has an extremely restricted distribution[4]. In 1957, another population of animals resembling *L. hamiltoni* was discovered on Maud Island in Pelorus Sound[5] and the

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following description applies to frogs from this locality only. The report is based on material from 3 adult females and 1 adult male.

Material and methods. The body lengths (snout to vent) ranged from 38–47 mm and the weights from 5.7–7.0 g. All the frogs were given i.p. injections of colchicine (0.5 ml, 0.01–0.05%) 12 h before they were killed and processed. As previously described for *L. hochstetteri* and *L. archeyi*[2], intestinal and testis squashes were prepared and cultures were made of kidney and lung tissue. These were incubated on collagen at 20–25 °C. The medium was based on that of WOLF and QUIMBY[6] but was prepared by combining the appropriate salts with 6.0 ml/l of Eagle's M.E.M. amino acid solutions A + B, vitamins, L-glutamine and non-essential amino acids (Commonwealth Serum Laboratories, Melbourne) together with 14% foetal calf serum. Whole egg ultrafiltrate was omitted. Cultures were allowed to grow for 3–4 weeks before metaphase spreads were prepared[2]. Karyotype measurements were based on 10 spreads from squash preparations and cultured cells and included material from all 4 specimens. Chromosome counts were made from more than 40 spreads.

Somatic chromosomes of *L. hamiltoni*. 9 pairs of chromosomes were present (Figure 1 and Table). Only the smallest pair was acrocentric and with this pair were associated secondary constrictions and small, terminal satellites. Microchromosomes were absent. The karyotype of *L. hamiltoni* resembled that of *L. archeyi* (Figure 2) in total chromosome number, overall pattern and number and position of acrocentric chromosomes. Some differences were found in the relative lengths of specific chromosomes[4,5] but the most obvious divergence was the location of the secondary constrictions.

Meiotic stages. After examination of numerous squashes of testes from a mature male, a lone primary spermatocyte at late prophase of meiosis I was found with 5 large and 4 medium-sized bivalents (Figure 3). Careful microscopic examination of bivalent orientation revealed that the configuration near the arrow in Figure 3 represented the overlapping of 2 medium-sized bivalents and not an interstitial chiasma of a large bivalent. Each of the 5 large bivalents had 2 terminal or near terminal chiasmata. One bivalent was already in a ring form, the others were at various stages of ring formation. Three of the 4 medium-sized bivalents also showed terminal chiasmata and a ring form, while the remaining one had the typical cross shape resulting from a single interstitial chiasma.

Discussion. The present report emphasizes the divergence of karyological pattern between *L. hochstetteri* on the one hand and *L. hamiltoni* and *L. archeyi* on the other. The differences between the karyotypes of *L. archeyi* and *L. hochstetteri* have already been enumerated and discussed[2] and the greater resemblance of the karyotype of the latter species to that of *Ascaphus* has been recorded[2]. The present chromosome study supports previous evidence

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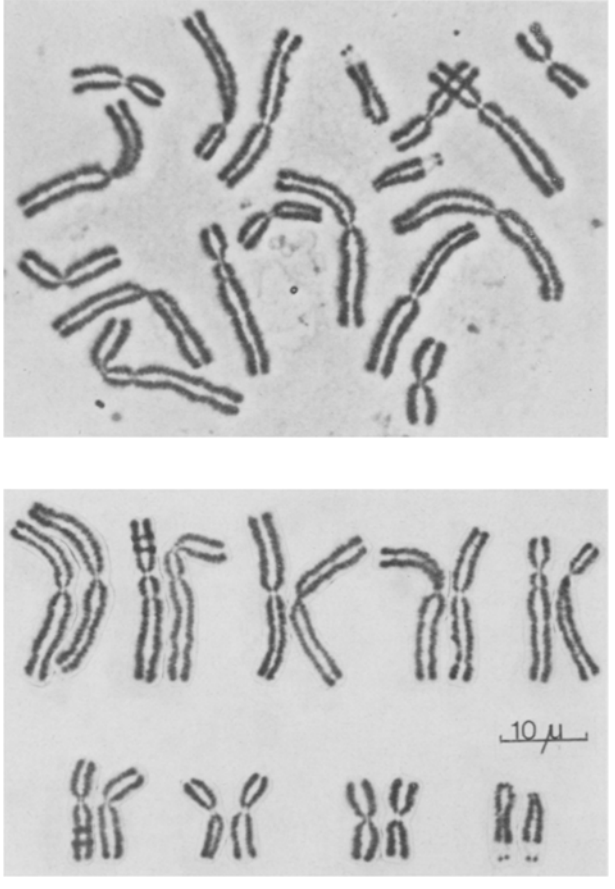

Fig. 1. a) *L. hamiltoni*. Metaphase spread prepared from cultured kidney cells of adult female. b) Karyotype from same spread.

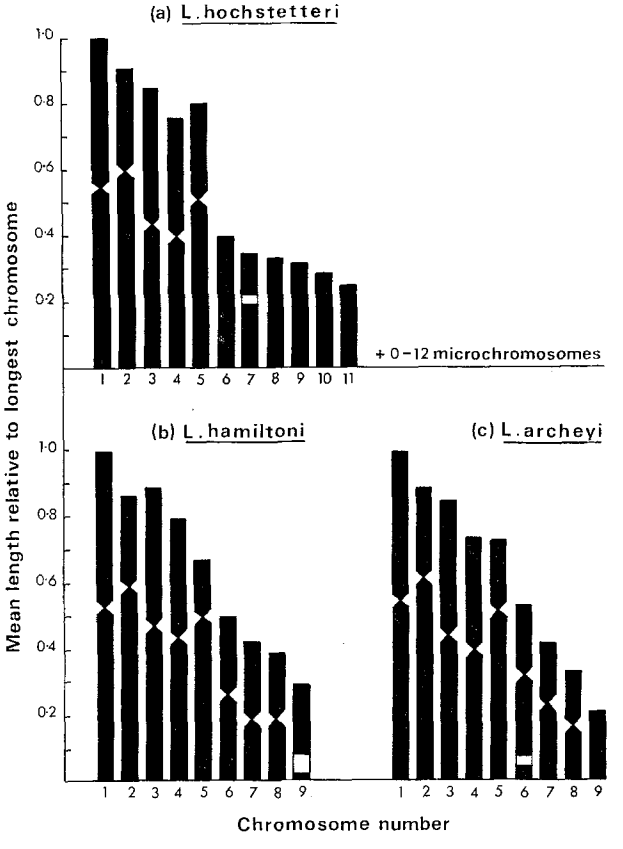

Fig. 2. Comparison of karyotypes of 3 species of *Leiopelma*. The linear order of metacentrics and submetacentrics has been standardized throughout, regardless of specific variations in relative lengths. Figures a) and c) have been redrawn after STEPHENSON, ROBINSON and STEPHENSON[2].

from external features and skeletal anatomy[5] that *L. archeyi* and *L. hamiltoni* have a closer affinity with each other than either does with *L. hochstetteri*. A neotenic basis for the apparent relationship between *L. archeyi* and *L. hamiltoni* has previously been suggested[5].

The degree of importance that should be attached to variations in relative lengths of specific chromosomes of *L. archeyi* and *L. hamiltoni* is questionable at this stage in view of the limited and suboptimal preparations on which the measurements of *L. archeyi* chromosomes were of necessity based[2]. It is desirable that further material of this species should be examined, particularly with regard to the detailed morphology of the chromosomes bearing the secondary constrictions. On present evidence (Figure 2), it appears to be theoretically feasible to derive a karyotype very similar to that of *L. archeyi* by postulating a translocation of the secondary constriction and telomere from Pair 9 of *L. hamiltoni* (Figure 2) to Pair 6 of the same species (Figure 2). Whatever the evolutionary basis of the apparent relationship between *L. archeyi* and *L. hamiltoni* may have been, it is now obscured by the total geographical separation of these 2 species. However, the combined information now available regarding the chromosome patterns of *Leiopelma* suggests that the time may now be appropriate for a taxonomic reassessment of the genus as a whole, combining evidence from all available areas of investigation.

Characteristics of early meiosis in male anurans have been tabulated by MORESCALCHI[7]. Those of the Ascaphidae and Discoglossidae are listed as being generally different from those of 'higher' anuran families. Diakinesis in *L. hamiltoni* appears to conform with the pattern for other ascaphids and discoglossids in some respects, but also has similarities to that of the more advanced families. The scarcity of diakinesis stages supports the contention that this stage is short in ascaphids and discoglossids. On the other hand, the possession of 2 terminal chiasmata in most bivalents in *L. hamiltoni* is an exception to the generalization that ascaphids and discoglossids have more than 2 chiasmata in large bivalents and that chiasma terminalization is normally never total. MORESCALCHI[7] referred to 'the presence, but not constant' of ring bivalents in *Discoglossus* and less commonly in *Alytes*. *L. hamiltoni* can be added to this group, although it should be noted that such an inclusion is based on a single spread.

MORESCALCHI[7–9] has suggested that bivalent morphology and behaviour of ascaphids and discoglossids represent primitive anuran conditions similar to the urodelan type. In urodeles the number, localization and terminalization of chiasmata between and even within species may vary and it would appear that there is also variation in these aspects of male meiosis in the genus *Leiopelma*.

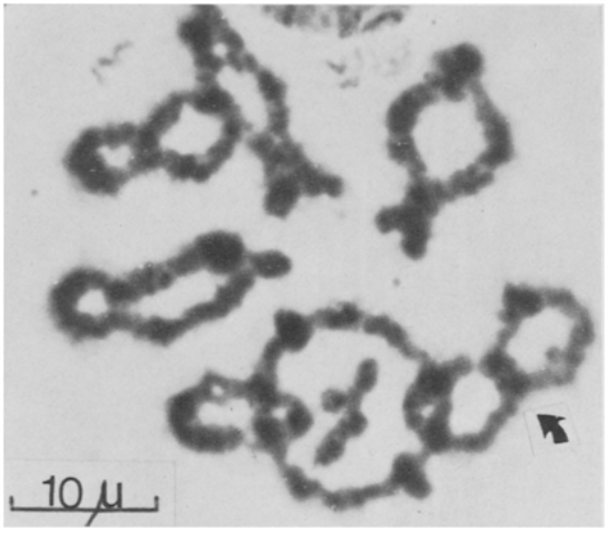

Fig. 3. Late prophase (diakinesis) of first meiotic division from testis squash of *L. hamiltoni*. The arrow indicates an area of overlapping of 2 medium-sized bivalents.

Résumé. Le caryotype de la grenouille couramment classée sous le nom de *Leiopelma hamiltoni* MCCULLOCH, de l'île de Maude, en Nouvelle Zélande, est décrit pour la première fois. A l'état diploïde, le nombre des chromosomes est de 18. La plus petite des paires est acrocentrique et présente de petits satellites terminaux. On n'observe pas de microchromosomes. Pendant la diacinèse de la meïose mâle on peut observer des chromosomes bivalents avec 2 chiasmas terminaux. L'évidence caryologique est en accord avec les indications précédentes concernant l'aspect extérieur et l'anatomie du squelette: *L. hamiltoni* ressemble davantage à *L. archeyi*, qu'à *L. hostetteri*. On suggère la nécessité d'une réévaluation taxonomique du genre.

E. M. STEPHENSON[10], E. S. ROBINSON and N. G. STEPHENSON[11]

School of Biological Sciences, A08, The University of Sydney, Sydney (N.S.W. 2006, Australia); and School of Biological Sciences, Macquarie University, North Ryde (N.S.W. 2113, Australia), 4 June 1974.

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Activity of Some Juvenoids in Chironomid Larvae

LAUFER et al.[1,2] reported that synthetic C_{18} *Cecropia* juvenile hormone and a mixture of derivatives of the farnesenic acid prevent metamorphosis in chironomids. The present study extends their observations and compares activities of some compounds which have been described as potent juvenoids for various Diptera.

Our tests were performed on last instar larvae of *Chironomus annularis* Meig. and *Ch. dorsalis* Meig., which were collected from an outdoor water container in Prague[3]. Groups of 10–15 larvae were kept in 50 ml of tap water in Petri dishes (diameter 12 cm) at room temperature. The larvae were fed with nettle powder and the water was