Chromosoma (Berl) (1988) 97:55–70

CHROMOSOMA
© Springer-Verlag 1988

# Cytogenetics of the endemic New Zealand frog, *Leiopelma hochstetteri*: extraordinary supernumerary chromosome variation and a unique sex-chromosome system

**David M. Green**
Redpath Museum, McGill University, Montréal, Québec H3A 2K6, Canada

**Abstract.** Cytogenetic data from 6 populations demonstrated unusual supernumerary chromosome variation in the primitive frog, *Leiopelma hochstetteri*. Frogs from the Coromandel Peninsula of the North Island of New Zealand averaged very high numbers of supernumerary chromosomes while individuals from other populations outside of the Coromandel region rarely had more than 1 distinctive supernumerary chromosome found only in females. The maximum number observed was 16 supernumeraries, present in 1 individual from Mt. Moehau. Supernumeraries showed meiotic instability as they failed to pair during prophase I in spermatocytes. In lampbrush preparations from oöcytes, supernumeraries appeared as univalents or as highly unusual stellate aggregations consisting of up to 7 chromosomes joined at their telomeres. Lateral loops on lampbrush supernumeraries indicated transcriptional activity. Contrary to a previous hypothesis, high supernumerary chromosome numbers in *L. hochstetteri* were not correlated with meiotic abnormalities. Neither were supernumerary chromosomes correlated with variations in heterochromatin distribution in the regular chromosomes. Rather, heterochromatin distribution was shown to vary geographically between populations. Sex determination in *L. hochstetteri* was found to be through a supernumerary, univalent W chromosome. Females in all populations invariably had 1 distinctive supernumerary chromosome not present in males. This chromosome could be distinguished from other supernumerary chromosomes by distinctive C-banding patterns and larger size. The W chromosome has undergone more rapid evolutionary change than the autosomes. Both telocentric and metacentric iso-chromosome forms were found in most populations. Heterochromatin distribution on the W chromosome varied between populations, from very little heterochromatin restricted to the centromere in Coromandel populations to an almost completely heterochromatic W chromosome among frogs from the East Cape region. In lampbrush preparations, the W chromosome was morphologically distinct from other supernumeraries. Loss of a Z chromosome leading to a univalent sex-determining W chromosome is difficult to explain through prevailing theories of sex-chromosome differentiation. The 0W♀/00♂ sex-determination system of *L. hochstetteri* appears to be unique among animals.

## Introduction

Recent chromosomal studies of the endemic New Zealand frog, *Leiopelma hochstetteri*, have indicated the occurrence of a number of unusual cytogenetic phenomena in this species (Green et al. 1984a, 1987). The basic karyotype consists of 5 pairs of metacentric chromosomes and 6 pairs of smaller telocentric chromosomes, giving $2n=22$. A prominent secondary constriction is invariably present on chromosome 7. However, as first recognized by Stephenson et al. (1972), *L. hochstetteri* also possesses variable numbers of supernumerary chromosomes in its karyotype. These supernumerary, or B, chromosomes are usually much smaller than the normal autosomes but, nevertheless, are not all uniform in size or shape (Green et al. 1987).

The three species of the genus *Leiopelma* are among the most phylogenetically primitive of living frogs (Duellman and Trueb 1986). They are remnants of a much larger and more widespread group of New Zealand endemics, including three extinct subfossil *Leiopelma* species (Worthy 1987). *L. hochstetteri* is found only in scattered localities on the North Island and on Great Barrier Island (Bell 1982). Although *L. hochstetteri* is both more widespread and more abundant than either of its two congeners, *L. archeyi* and *L. hamiltoni*, all three species of *Leiopelma* are strictly protected by the New Zealand Government (Bell 1986). Populations of *L. hochstetteri* have suffered greatly from destruction of stream habitats because of logging and silviculture operations and by the clearing of land for pastures (Bell 1985).

*L. hamiltoni* and *L. archeyi* have karyotypes that are extremely similar to each other (Stephenson et al. 1972, 1974; Green 1988; Green and Sharbel 1988) and, unlike *L. hochstetteri* with its autosomal set of $2n=22$ chromosomes, have only $2n=18$ chromosomes and nuclear organizer regions (NORs) on near-terminal secondary constrictions on the smallest chromosomes (Green 1988; Green and Sharbel 1988). Green (1988) has demonstrated heteromorphic Z and W sex chromosomes in *L. hamiltoni*.

All karyotypic studies of *L. hochstetteri*, and, indeed, most studies of any kind on this frog, have been hampered by the difficulty of obtaining specimens. Morescalchi (1967) presented chromosomal results from only 2 female specimens while Stephenson et al. (1972) had an additional 4 specimens. Green et al. (1984a) were able to examine 6 more frogs and later (Green et al. 1987) added a further 6 specimens. These 18 frogs were collected from only 3 different localities. Despite the low numbers of animals examined, Green et al. (1987) documented 9 different cytotypes in *L. hochstetteri*. These included 1 triploid individual (Green et al. 1984a) and karyotypes having from 0 to 12 supernumeraries (Morescalchi 1967; Stephenson et al. 1972; Green et al. 1984a, 1987).

Even with this small and piecemeal sampling, a number of trends appeared to exist (Green et al. 1987). There seemed to be geographic differentiation between populations as to the number of supernumerary chromosomes present per individual, possibly along a latitudinal gradient. Noticeable differences in C-band heterochromatin distribution between populations seemed to correlate with the numbers of supernumerary chromosomes present per individual. This would support the hypothesis that supernumerary chromosomes may arise from centromere fragments (Jones and Rees 1982). One male with a high number of supernumerary chromosomes could be examined for meiotic figures and was found to be highly abnormal, exhibiting either asynapsis or chiasma failure among the telocentric elements. Finally, data presented by Green et al. (1987) indicated a possible sexual difference in the occurrence of a supernumerary chromosome in one population. However, because of the limited numbers of available specimens of *L. hochstetteri*, previous findings remained only indicators of possible trends in what appeared to be unusual chromosome variation in this frog.

In 1987, the New Zealand Wildlife Service granted me permission to collect additional frogs for study. This paper is based upon results obtained from 49 specimens collected from 6 localities in New Zealand. These data clarify previous data and further illustrate the unusual cytogenetic variability present in *L. hochstetteri*.

## Materials and methods

Specimens of *L. hochstetteri* were captured on the North Island of New Zealand in January of 1987 and were transported live to Montreal for study. The frogs were collected from 6 localities in New Zealand (Fig. 1) as follows: Big Omaha northeast of Warkworth (6 females, 4 males); Mangatangi Dam in the Hunua Mountains west of Kaiaua (4 females, 1 male); Tapu (5 females, 4 males), Tokatea Ridge (7 females, 3 males) and Mt. Moehau (3 females, 2 males) on the Coromandel Peninsula; and Toatoa in the East Cape region (5 females, 5 males). I had actually collected 10 frogs from Tapu but one frog died before it could be used, leaving the above 49 specimens whose chromosomes could be examined. Big Omaha is very close to Dome Valley, the site where frogs had been obtained for the studies by Morescalchi (1967), Stephenson et al. (1972) and Green et al. (1984a, 1987). All specimens are deposited in the National Museum of Natural Sciences, Ottawa, Canada (NMNS lot nos. 29587–29590 and 29592–29596).

Chromosome squash preparations were made according to Bogart's (1981) method using corneal epithelium or Kezer and Sessions' (1979) technique using gut epithelium and testis. Best results were obtained using cornea. All preparations were examined unstained with phase contrast using a Zeiss photomicroscope. Good chromosome spreads were photographed. Chromosomes were measured from photographic prints using the system described by Green et al. (1984b). Nomenclature for chromosomes is based on that suggested by Levan et al. (1964), as modified by Green et al. (1980).

Slides were C-banded to demonstrate constitutive heterochromatin using the technique described by Schmid (1978). Slides baked for up to 2 days at 60° C gave good results with a 5.5–min barium hydroxide treatment at 40° C followed by 1 h incubation in 2 × SSC at 60° C and staining in 8% Giemsa in pH 7.2 phosphate buffer. (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate.)

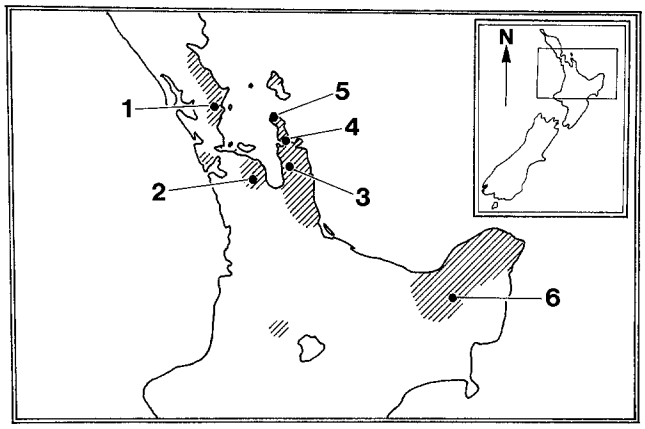



**Fig. 1.** Map of the central portion of the North Island of New Zealand (*inset*) showing the range of *Leiopelma hochstetteri* (*cross-hatching*) and localities mentioned in the text. *1* Big Omaha and Dome Valley; *2* Managtangi Dam; *3* Tapu; *4* Tokatea Ridge; *5* Mount Moehau; *6* Toatoa

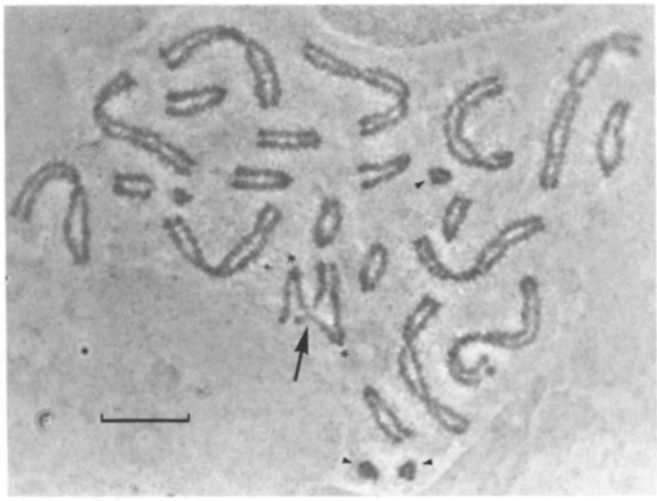


**Fig. 2.** Metaphase spread from corneal epithelium of *Leiopelma hochstetteri* female (NMNS 29594-4) from Managtangi. A somatic chromatid translocation between two different telocentric autosomes is evident (*large arrow*). *Asterisks* indicate the centromeres of these chromosomes. This specimen also has three supernumerary chromosomes (*small arrowheads*). Bar represents 10 μm

NORs were demonstrated using Hsu's (1981) "quick gelatine" silver staining method. Fresh slides that had been dehydrated in 95% ethanol and air-dried produced the best results but old, baked slides could also be used if they were pre-treated with a 95% ethanol bath. Two drops of developer (0.5 ml formic acid in 50 ml of 2% gelatine solution) and 4 drops of 50% silver nitrate solution were put on the slide, carefully mixed and covered with a cover slip. Slides were incubated at 60° C for 5 min 10 s then rinsed in distilled water and air-dried.

Lampbrush preparations from oöcytes were prepared by Dr. H.G. Callan, with the author assisting. The methods were the same as described by Callan et al. (1987) to obtain lampbrush chromosomes from *Xenopus*. One female from each of the Big Omaha, Mangatangi and Tapu populations

was used. Portions of ovary were surgically removed from these frogs; the frogs were later used to obtain mitotic preparations confirming the numbers of supernumeraries present in each. Useful results were obtained from three to five oöcytes prepared from each frog. The best lampbrush results were from fairly small oocytes of about 0.7 to 1.0 mm in diameter. Oöcytes that were too small were found to have extremely fluid nuclear sap and were very difficult to manipulate. Larger oöcytes, of around 3.0 to 3.5 mm, were much easier to use as they had quite gelled nuclear sap but the chromosomes were well past the peak of lampbrush activity and had greatly reduced lateral loops. In these larger cells, a grey patch on the surface of the otherwise unpigmented oocyte usually identified the position of the germinal vesicle but isolating the germinal vesicle from these very yolky eggs was rather difficult. The lampbrush preparations were centrifuged and fixed in buffered formalin. They were stained with Coomassie Brilliant Blue R250 and mounted in Canada Balsam. Later, the preparations were restained in iron-haematoxylin as described by Callan et al. (1987) and remounted. These permanent preparations were examined in detail and photographed.

## Results

Results sufficient to count all regular and supernumerary chromosomes were obtained from all 49 frogs available. The regular set of $2n=22$ chromosomes was as previously described by Stephenson et al. (1972) and Green et al. (1984a, 1987). I saw no additional variation in these chromosomes in unstained preparations except for one unusual observation. One cell from a female frog from Managatangi featured a chromatid translocation involving two unequally sized telocentric chromosomes (Fig. 2). This anomaly appeared to be strictly somatic as no other cells from this individual, including oöcytes, showed this odd configuration.

### *Chromosome banding of autosomes*

C-bands and NOR bands were obtained from individuals of both sexes from all populations considered. The NOR in all specimens was clearly associated with the secondary constriction on chromosome 7 (Fig. 3). No significant variation in NOR staining was seen.

C-banding patterns from Big Omaha specimens were as described by Green et al. (1984a, 1987) in specimens from the nearby site of Dome Valley. All chromosomes in these frogs, including the large metacentrics, C-banded heavily at their centromeres (Fig. 4a). The specimens from Mangatangi exhibited a pattern like that of the Big Omaha/Dome Valley frogs. The pattern for Coromandel Peninsula specimens was as described by Green et al. (1987). In these animals, centromeric heterochromatin stained strongly in the telocentric and supernumerary chromosomes, but weakly or not at all in the large metacentric chromosomes (Fig. 4b). Interstitial C-bands, not associated with secondary constrictions, were evident in two pairs of telocentric chromosomes. As described by Green et al. (1984a, 1987), the secondary constriction on chromosome 7 was C-band positive in the three Coromandel samples, and those from Big Omaha and Managatangi (Fig. 4a, b). In the frogs from Toatoa, centromeric C-band heterochromatin was distributed in a manner like that of the Coromandel Peninsula

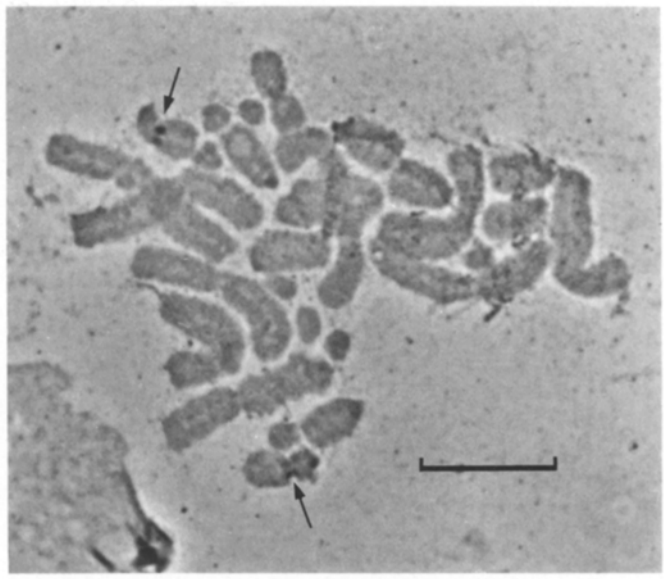


**Fig. 3.** Nuclear organized region (NOR) banding in a female *Leiopelma hochstetteri* (NMNS 29592-5) from Tokatea. The NORs are evident at the sites of the secondary constrictions on chromosomes pair 7 (*arrows*). This frog has seven supernumery chromosomes. Bar represents 10 μm

frogs, (i.e. little heterochromatin on the large metacentrics compared with the telocentrics and supernumeraries) but the secondary constriction site did not feature C-band heterochromatin (Fig. 4c).

### *Spermatocyte meiosis*

Meiotic preparations were obtained from the testes of males with various numbers of supernumerary chromosomes (Fig. 5). There was no discernible effect on the behaviour of the regular chromosomes that could be correlated with the presence or absence of supernumeraries. All autosomes paired and exhibited numerous, interstitial chiasmata in diakinesis, as seen and described by Stephenson et al. (1972). Supernumerary chromosomes, where present, did not pair and appeared not to be involved in meiotic events. Meiotic anomalies of the type documented in 1 frog by Green et al. (1987) were not seen in any of 19 male frogs.

### *Numbers of supernumerary chromosomes*

Besides the 22 regular chromosomes found in all frogs, up to 16 supernumerary chromosomes were present per individual. The supernumerary chromosomes were mitotically stable. As shown and explained earlier by Green et al. (1987), among all the cells examined for any given individual, a distinct modal number of supernumeraries could always be counted which was equal, or close to, the maximum number counted per cell. Thus any deviations from the mode could be ascribed to artefact.

Distinct differences in the numbers of supernumerary chromosomes were found between populations and sexes (Table 1). From Big Omaha and Toatoa, all females had a single supernumerary univalent (Fig. 6) while all males from these populations were without supernumeraries. Among the 5 frogs from Mangatangi, the 4 females had either 2, 3 or 4 supernumeraries (Fig. 2) while the single male had none.

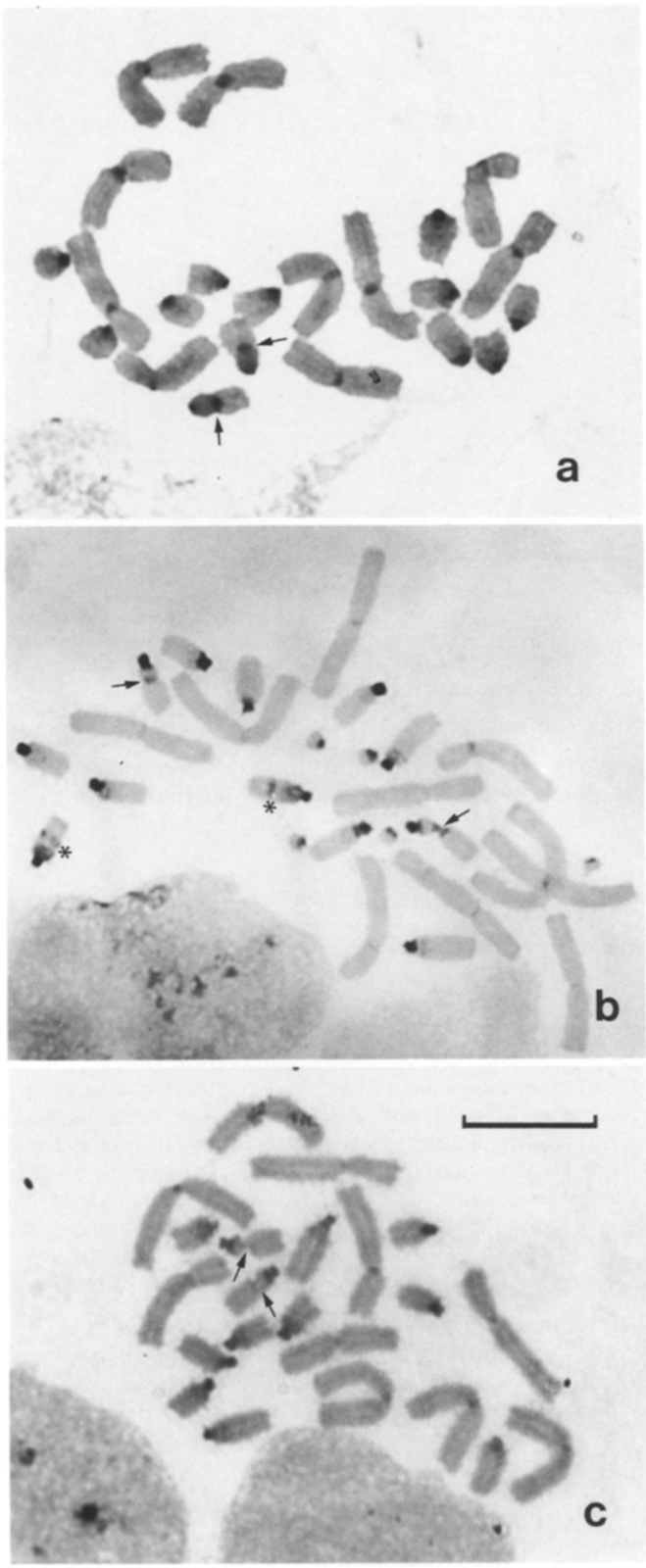



**Fig. 4a–c.** C-banded chromosomes of *Leiopelma hochstetteri* males. **a** A male (NMNS 29595-2) from Big Omaha has no supernumerary chromosomes and exhibits strong C-banding at the centromeres of all autosomes, including the large metacentric elements. The secondary constriction sites on chromosome pair 7 (*arrows*) also show heterochromatin. **b** Heterochromatin is reduced at the centromeres of the metacentrics in a male with five supernumeraries (NMNS 29589-9) from Tapu, although the telocentrics stain heavily at their centromeres, as do the secondary constrictions (*arrows*). Interstitial C-bands identify a pair of telocentrics (*asterisks*). **c** In a male (NMNS 29587-4) from Toatoa, heterochromatin is reduced or lacking at the centromeres of the large metacentrics, as well as at the secondary constriction sites (*arrows*). Bar represents 10 μm

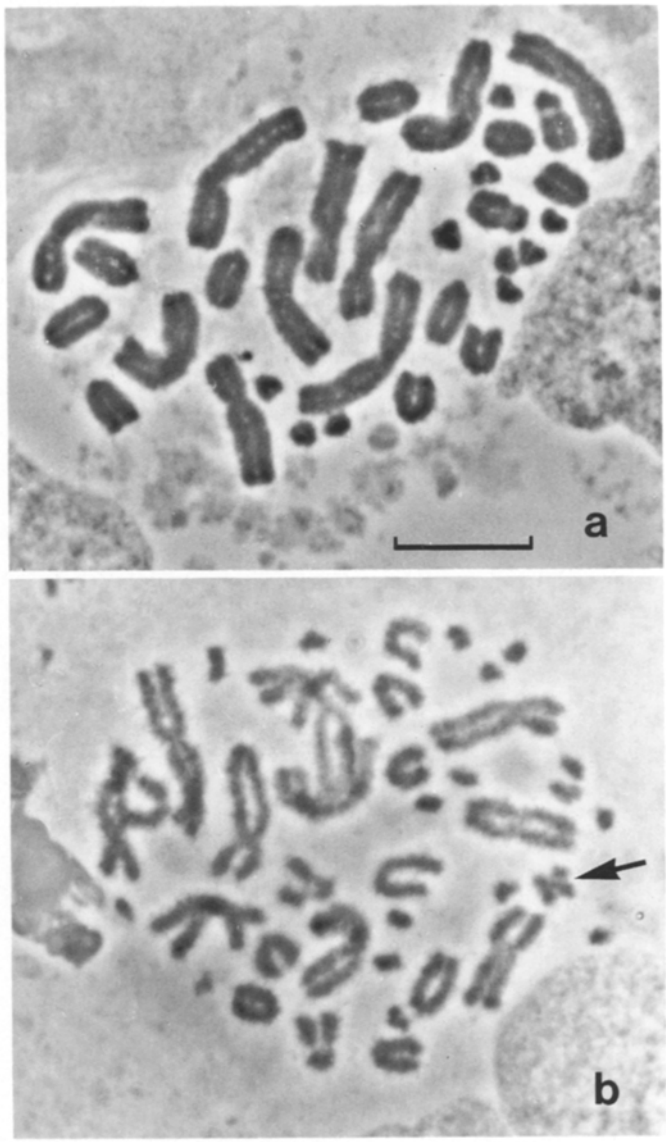



**Fig. 5a, b.** Unstained mitotic chromosomes of *Leiopelma hochstetteri* from Mt. Mochau illustrating extremely high numbers of supernumerary chromosomes. **a** Male (NMNS 29593-4) with 10 supernumeraries; **b** female (NMNS 29593-1) with 16 supernumeraries, 1 of which (*arrow*) is a metacentric chromosome with arms visibly longer than any of the other 15 small, telocentric supernumeraries. Bar represents 10 μm

The populations on the Coromandel Peninsula all had much higher numbers of supernumerary chromosomes per individual than the other populations. All frogs from the 3 Coromandel populations possessed supernumeraries, except for 1 male from Tokatea (Table 1). Frogs from Tokatea exhibited a mean of 4.2 supernumeraries each (range=

**Table 1.** Total numbers of supernumerary chromosomes found in individual *Leiopelma hochstetteri* in six populations

| Population | Numbers of supernumeraries | Mean | Source[a] |
|---|---|---|---|
| Big Omaha/Dome Valley | | | |
| Males (6) | 0 (6) | 0 | b, c, e |
| Females (15)[b] | 1 (13), 2 (1), 12 (1) | 1.8 | a, c, d, e |
| Mangatangi Dam | | | |
| Males (1) | 0 (1) | 0 | e |
| Females (4) | 2 (2), 3 (1), 4 (1) | 2.8 | e |
| Tapu | | | |
| Males (7) | 5 (1), 6 (2), 7 (2), 9 (1), 10 (1) | 7.2 | d, e |
| Females (6) | 6 (2), 7 (2), 10 (1), 13 (1) | 7.6 | d, e |
| Tokatea Ridge | | | |
| Males (6) | 0 (1), 2 (2), 3 (1), 4 (1), 5 (1) | 3.1 | b, e |
| Females (7) | 4 (4), 5 (1), 6 (1), 11 (1) | 5.5 | b, e |
| Mount Moehau | | | |
| Males (2) | 10 (1), 12 (1) | 11.0 | e |
| Females (3) | 12 (1), 13 (1), 16 (1) | 13.7 | e |
| Toatoa | | | |
| Males (5) | 0 (5) | 0 | e |
| Females (5) | 1 (5) | 1.0 | e |

[a] Compiled from all known sources: *a* Morescalchi (1967); *b* Stephenson et al. (1972); *c* Green et al. (1984a); *d* Green et al. (1987); *e* present study

[b] The specimen with 2 supernumeraries is a triploid female described by Green et al. (1984); the specimen with 12 supernumeraries was described by Morescalchi (1967) but it may not have originated from this population. The mean is calculated exclusive of the triploid but including the latter individual

Types of supernumerary chromosomes as discussed in this paper are not distinguished. Numbers of individuals are *in parentheses*

0–11), frogs from Tapu averaged 7.4 supernumeraries (range = 5–13) while the frogs from Mt. Moehau, with astounding numbers of supernumeraries (Fig. 7), showed a mean of 12.6 supernumeraries per individual (range = 10–16). This appears to be, by far, the highest number of supernumerary chromosomes recorded in any vertebrate (Jones and Rees 1982). The next highest number in a vertebrate is 10, seen in the rodent, *Perognathus baileyi* (Patton 1977). Among all animals, 16 or more supernumeraries has been recorded only in 2 insects (Boyes and Van Brink 1967; Sannomiya 1974).

Females generally had higher numbers of supernumerary chromosomes than did males from the same population. This is obvious in the Big Omaha, Toatoa and Mangatangi populations (Table 1) but this observation also holds true in the three Coromandel Peninsula populations, where males also had some supernumeraries.

### *Types of supernumerary chromosomes*

The supernumerary chromosomes were not all the same size nor configuration. This was observed by Green et al. (1987) but even more variation was found in the present samples. Two main classes of supernumerary chromosomes are distinguishable which I designate *s* and *W*. Each of these classes has both telocentric and metacentric forms (i.e. *ts, ms, tW, mW*) that can be observed in unstained preparations. Further differentiation of types is possible using chromosome bands.

A small, *ts* chromosome (telocentric supernumerary) found among both sexes in Coromandel Peninsula populations (Fig. 7) and among females from Mangatangi (Fig. 2) was usually present in multiple, equal-sized copies. This *ts* chromosome accounts for the high numbers of supernumeraries in the Tapu, Tokatea and Mt. Moehau populations of the Coromandel Peninsula as all other supernumerary chromosome types seen were present only as single univalents. A small metacentric *ms* chromosome was found in a single male from Tapu by Green et al. (1987). I did not observe such a chromosome in the present study. Green et al. (1987) postulated that it was an iso-chromosome version of the more common telocentric supernumeraries (i.e. *ts* chromosomes) or else was a fusion of two supernumeraries.

The many forms of *W* chromosomes were observed only in females. They were most obvious in the Big Omaha and Toatoa samples (Fig. 6) where they were the only supernumeraries present and so could not be confused with the

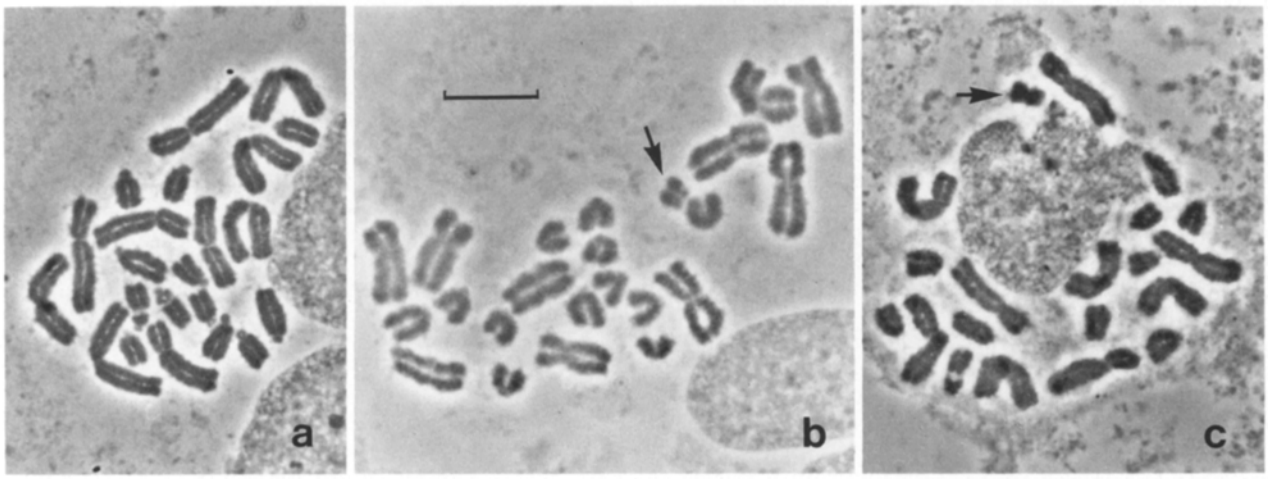



**Fig. 6a–c.** Mitotic chromosomes from three frogs from Toatoa. **a** As in all males from this population, this frog (NMNS 29587-4) has no supernumeraries present. **b** Female (NMNS 29587-1) with a single, telocentric, supernumerary *tW* chromosome (*arrow*) present. **c** Female (NMNS 29588-6) with a single metacentric supernumerary *mW* chromosome (*arrow*). Bar represents 10 μm

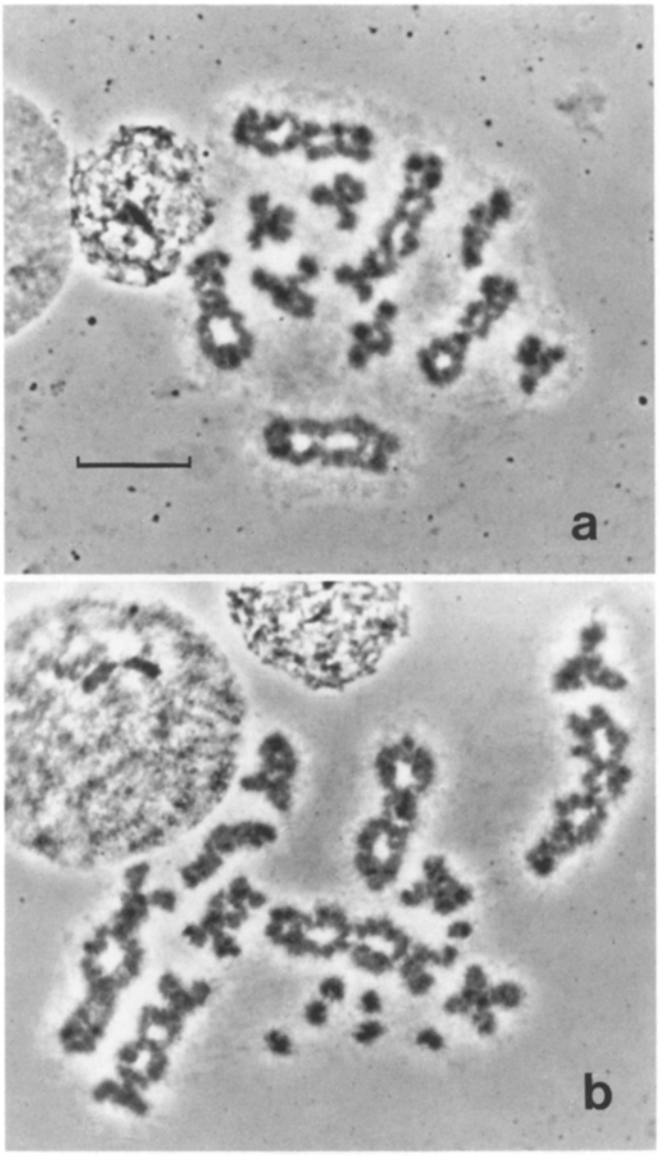



**Fig. 7a, b.** Spermatocyte meiosis in *Leiopelma hochstetteri*. **a** Diakinesis in a male (NMNS 29590-3) from Tokatea without supernumerary chromosomes. **b** Equivalent stage in a male (NMNS 29589-3) from Tapu with 7 supernumeraries. The supernumeraries are easily visible, small univalent structures among the 11 completely normal autosomal bivalents. Bar represents 10 μm

smaller *ts* supernumeraries of other populations. Telocentric *tW* supernumeraries were larger than *ts* supernumeraries by a factor of about 1.6×, as discussed by Green et al. (1987). All females from Big Omaha had telocentric *tW* supernumeraries but a metacentric iso-chromosome version, *mW*, was found among females from the other populations. This *mW* chromosome had not been observed in any

**Table 2.** Incidence of metacentric iso-W-chromosomes (*mW* chromosomes) among female *Leiopelma hochstetteri*

| Population | No. of females examined | No. of females with *mW* chromosome | Percentage of females with *mW* chromosome |
|---|---|---|---|
| Dome Valley/Big Omaha | 15 | 0 | 0 |
| Mangatangi | 4 | 1 | 25 |
| Tapu | 6 | 1 | 17 |
| Tokatea | 7 | 4 | 57 |
| Mt. Mochau | 3 | 3 | 100 |
| Toatoa | 5 | 4 | 80 |

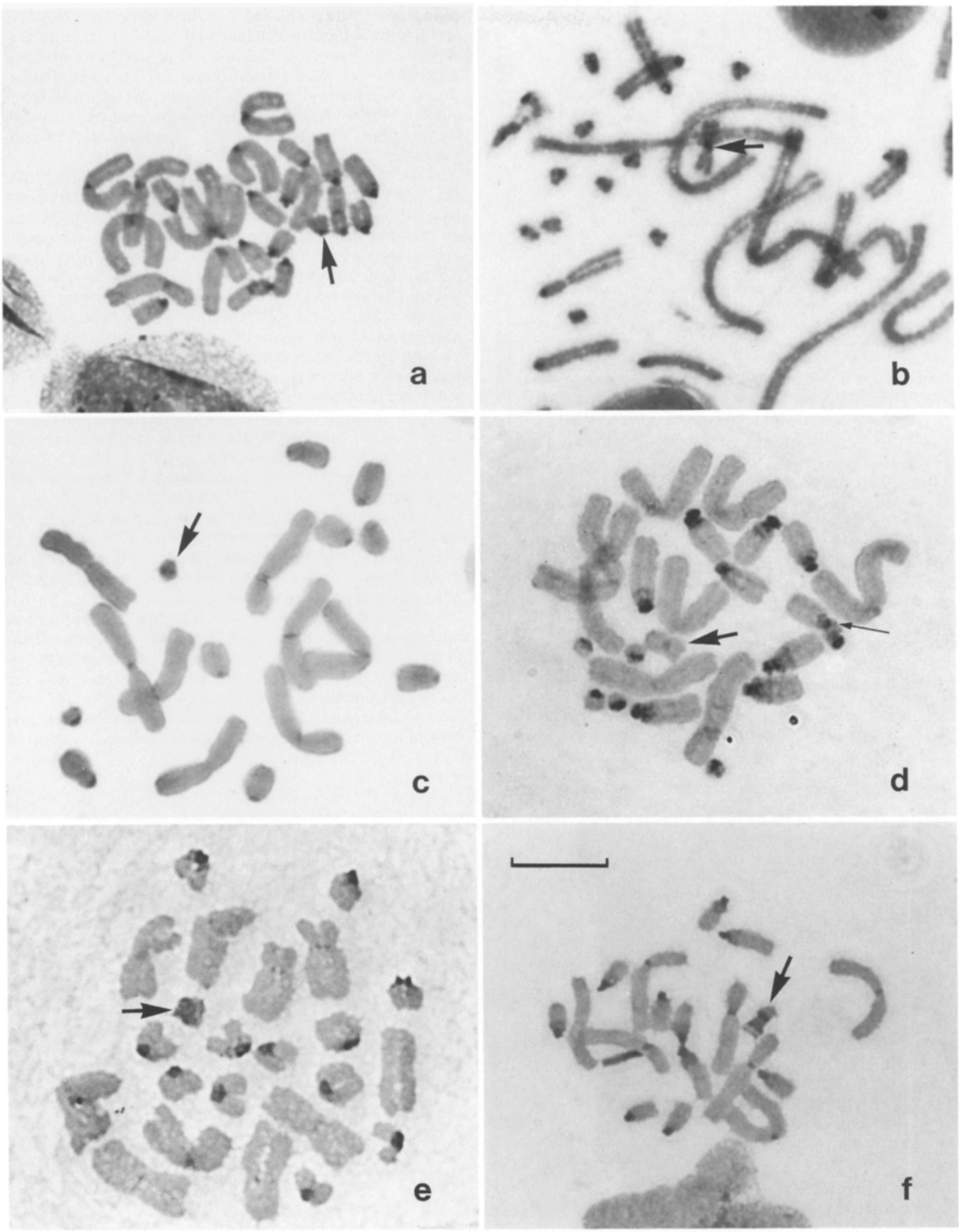
Compiled from all known sources (as indicated for each population in Table 1)

previous study of the chromosomes of *L. hochstetteri* and so it was surprising to find it in high frequency in some populations (Table 2). Either a *tW* or an *mW* was present in any individual female; no diploid individual had more than one of these chromosomes. The triploid female studied by Green et al. (1984a) which had two *tW* chromosomes had evidently developed from an oöcyte that had suppressed the second meiotic division.

The incidence of *mW* chromosomes, as opposed to *tW* chromosomes, varied between populations, with general trends towards increased frequency from northwest (Big Omaha) to southeast (Toatoa) and up the Coromandel Peninsula (Table 2). Absent from all Big Omaha females, an *mW* chromosome was present in 1 of 4 Managtangi females and 4 of 5 Toatoa females. Among frogs from the Coromandel studied here, 1 of 5 females from Tapu, 4 of 6 females from Tokatea, and all 3 females from Mt. Moehau had an *mW*.

Further distinction of *W* chromosome types relied upon considerable observed heterochromatin differences (Figs. 8, 9). Also, in each population except Tokatea, C-band patterns allowed the unequivocal distinction of *W* and *s* type supernumeraries. In females from Big Omaha, the $tW_1$ had heterochromatin restricted to the centromere in an amount similar to that seen in the autosomes (Fig. 8a). This was previously illustrated by Green et al. (1984a) in frogs from nearby Dome Valley. Both $tW_1$ and $mW_1$ chromosomes were observed among frogs from Tokatea. The heterochromatin in these chromosomes was virtually the same as in the co-occurring *ts* supernumeraries, making differentiation of the $tW_1$ difficult. Only the $mW_1$ was seen among frogs from Mt. Moehau (Fig. 8b). The $tW_2$ and $mW_2$ chromosomes from Managtangi females had prominent centromeric heterochromatin and an additional telomeric C-band (Fig. 8c). In the frogs from Tapu, the $tW_3$ and $mW_3$ had very limited amounts of C-band heterochromatin restricted to the centromere (Fig. 8d). The fact that the $tW_2$ from Tapu had much less heterochromatin than did the *ts* super-

**Fig. 8a–f.** C-banded chromosomes from female *Leiopelma hochstetteri* from different populations illustrating variation in W chromosomes. **a** A female (NMNS 29595-5) from Big Omaha has a $tW_1$ chromosome (*arrow*) which has a similar amount of heterochromatin to the rest of the chromosome set. **b** Female from Mt. Moehau with an $mW_1$ chromosome (*arrow*) which has heterochromatin evident at the centromere. **c** A female (NMNS 29594-3) from Managatangi with a $tW_2$ sex chromosome (*arrow*) in which two heterochromatin bands identify the centromere and telomere. **d** A females (NMNS 29589-2) from Tapu has a $tW_3$ sex chromosome (*large arrow*). Despite evidence of strong C-banding at the secondary constriction site (*small arrow*) and at the centromeres of the telocentrics and *ts* supernumeraries, heterochromatin is reduced or lacking at the centromere of the W chromosome. **e** A female (NMNS 29587-1)




from Toatoa with a highly heterochromatic $tW_4$ chromosome (*arrow*). **f** Another female from Toatoa (NMNS 29588-1) shows a highly heterochromatic, metacentric $mW_4$ chromosome (*arrow*). The heterochromatin is symmetrically distributed. All figures are at the same scale. Bar represents 10 μm

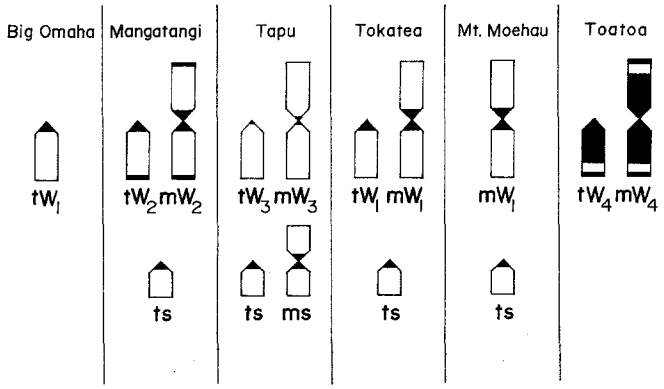



**Fig. 9.** Types of supernumerary chromosomes found in populations of *Leiopelma hochstetteri* (diagrammatic). *Black* indicates located of C-band heterochromatin on the chromosomes. Only the *ts* chromosome was found to occur in multiple copies (up to 15) per individual. The various *W* chromosomes are restricted to females. In all cases, *mW* chromosomes appear to be iso-chromosome versions of *tW* chromosomes found in the same population

numerary chromosomes was not noted by Green et al. (1987) who published C-banded preparations only from males. Finally, in female frogs from Toatoa, $tW_4$ and $mW_4$ chromosomes had greatly increased amounts of heterochromatin (Fig. 8e, f) which extended from the centromere region along most of each arm. In all of the various *mW* chromosomes, both arms had heterochromatin in the same relative positions as on the one arm of the corresponding *tW* chromosome from the same population (Fig. 9). Thus, in each case, the *mW* appeared as though it were an iso-chromosome version of a co-occurring *tW*.

*Lampbrush chromosomes*

Lampbrush preparations were made from 3 females: 1 from Big Omaha with 1 supernumerary *tW* (Fig. 10), 1 from Mangatangi with 1 *tW* and 2 *ts* supernumeraries (Fig. 11) and 1 from Tokatea which had a *tW* and 12 *ts* supernumerary chromosomes (Fig. 12). In good preparations, all bivalent autosomes could be accounted for. They featured assorted "landmarks" such as spheres and unusual loops (Callan 1986), but could not be isolated adequately enough for mapping to be attempted. The supernumerary chromosomes could usually be seen easily.

Like all other females from the same population, the Big Omaha female had a single, *tW* supernumerary (Fig. 10a). In the lampbrush state (Fig. 10b), the *tW* had an axis that was slightly denser than that in the autosomes and, as shown by Green et al. (1987) in a female from Dome Valley, had numerous lateral loops occurring along its length. The lateral loops were most prominent in a region near the telomere end of the chromosome. A small, granular loop structure was associated with the centromere. There were no other large, apparent landmarks. This chromosome was always univalent, not associating or synapsing with any other chromosome in the karyotype.

In the Mangatangi female, two sorts of small univalent chromosomes could be identified even in mitotic preparations (Fig. 11a). In the lampbrush condition (Fig. 11c), one of these univalent chromosomes had a configuration like the lampbrush *tW* in the Big Omaha female. It had lateral loops all along its length, but concentrated at the telomere, and had a granular structure associated with the centromere. This morphology identifies it as the *tW* chromosome in this female. The other two lampbrush supernumeraries were very similar to each other but differed markedly from the *tW*. Evidently *ts* chromosomes, they had prominent, fluffy-looking Giant Granular Loops (GGLs; Callan 1986) at the telomere end. A smaller granular structure was evident at the end opposite to the GGL associated with the dense centromere. Other landmarks were evident as well. Near to each end, smaller chromomere structures were evident along the axis. Lateral loops were largely restricted

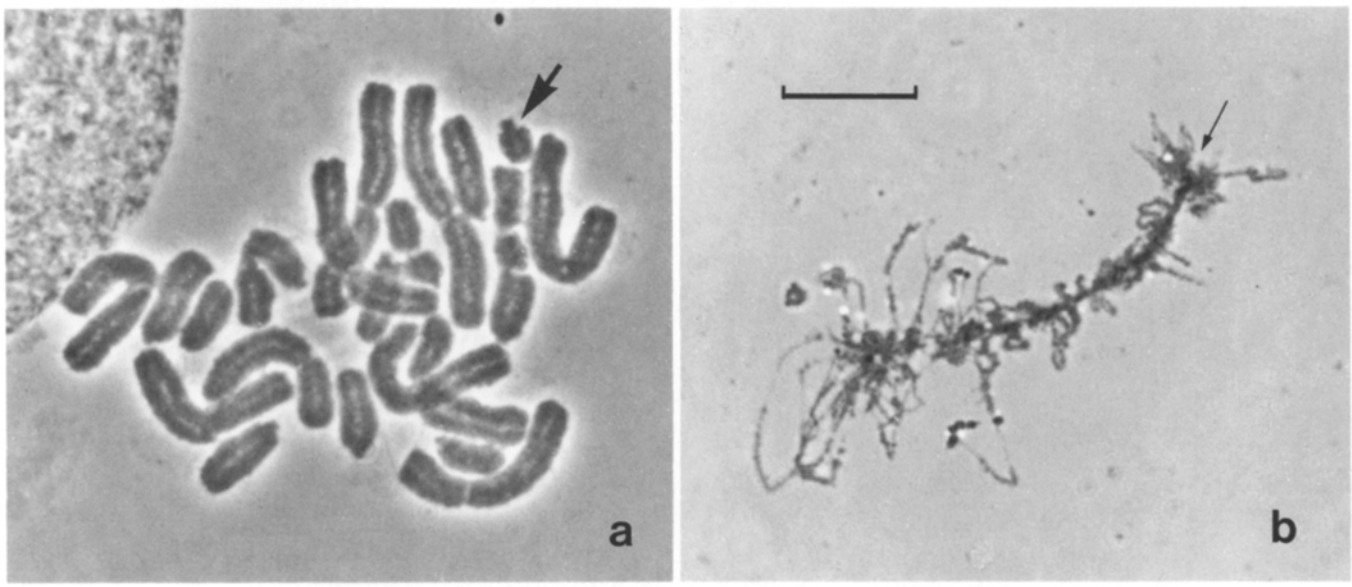



**Fig. 10a, b.** The W chromosome from a female *Leiopelma hochstetteri* (NMNS 29595-5) from Big Omaha in mitotic metaphase and lampbrush conditions. **a** The telocentric W chromosome is indicated (*arrow*) in a mitotic preparation. **b** Lampbrush preparation of the W chromosome from a 1.0 mm oocyte of the same individual, shown at the same scale. Numerous lateral loops indicate transcriptional activity, especially at the telomere end of the chromosome (*lower left*). An expanded structure is visible at the other end surrounding the dense centromere (*arrow*). There are no other evident landmark structures. Bar represents 10 μm

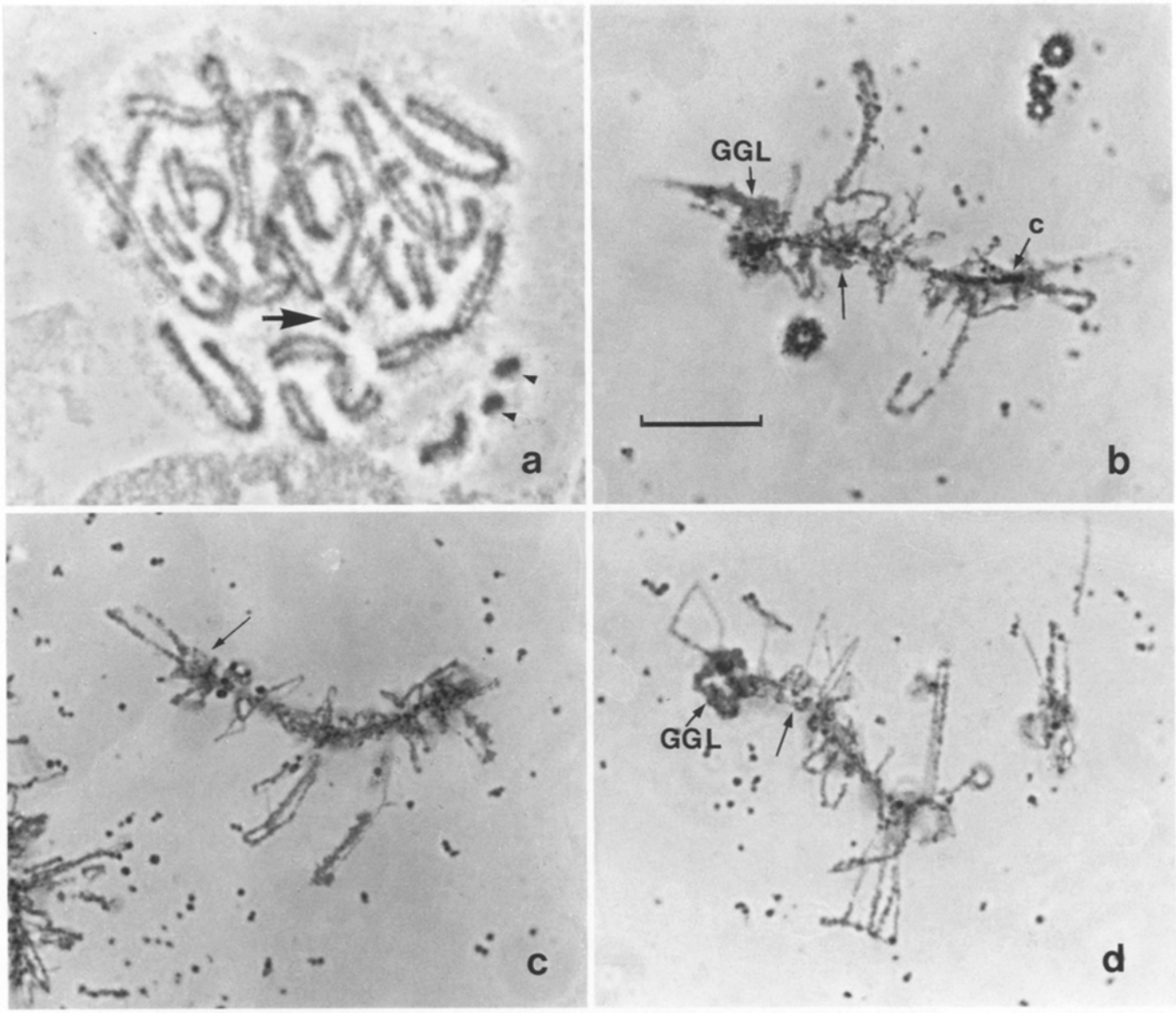



**Fig. 11a–d.** Mitotic chromosomes and lampbrush supernumerary chromosomes, at the same scale, from a female *Leiopelma hochstetteri* (NMNS 29594-4) from Mangatangi. **a** These mitotic chromosomes appear to be incompletely condensed and illustrate an apparent difference in size between the putative W chromosome (*large arrow*) and two *ts* supernumerary chromosomes (*small arrowheads*). **b, d** The two *ts* chromosomes in lampbrush condition from a 1.0 mm oocyte have identical landmarks. Both chromosomes have large Giant Granular Loops (*GGL*) at their telomere ends. Approximately one-fourth of the way along the axis is another granular loop structure (*arrow*). The centromere (*c*) is clearly visible in the chromosome in **b**. Dense, round objects are free nucleoli. **c** The lampbrush *tW* chromosome does not have the characteristic landmark features present in the *ts* supernumerary chromosomes from the same cell. It resembles the lampbrush W chromosome in Figure 10b from a Big Omaha female. An expansion is present at the centromere end (*arrow*) while there appears to be an increase in number of lateral loops at the other end (*right*). Bar represents 10 μm

to the central region of each chromosome. These *ts* chromosomes appeared singly in the oöcytes examined from this female.

In the female examined from Tokatea (Fig. 12), a single lampbrush univalent, evidently the *tW* chromosome (Fig. 12e), had a configuration quite like the single, supernumerary, univalent *tW* chromosomes identified in the Big Omaha and Managatangi females (Figs. 10b, 11c). This chromosome had small, lateral loops along its length but no large, prominent landmarks nor a GGL.

The 12 supernumery *ts* chromosomes of the Tokatea female, though, were in an astounding configuration (Fig. 12a). They appeared to associate by clumping at their telomere ends to form stellate aggregations, or "stars". The arms of the stars appeared to be quite fragile in the preparations obtained and some were apparently broken or folded. The different chromosomes making up the stars also exhibited differential amounts of condensation. However, the chromosome axes could be seen among the material of the central "nucleus" of each star so that the number of participating chromosomes could be counted. Up to 7 chromosomes at a time were observed in these clusters. In one cell, 2 stars of 5 and 7 *ts* chromosomes were seen while in another cell, 3 stars of 2, 4 and 6 *ts* chromosomes were seen (Fig. 12b, c, d). The total number of arms of all the lampbrush stars in any one nucleus added up to the total

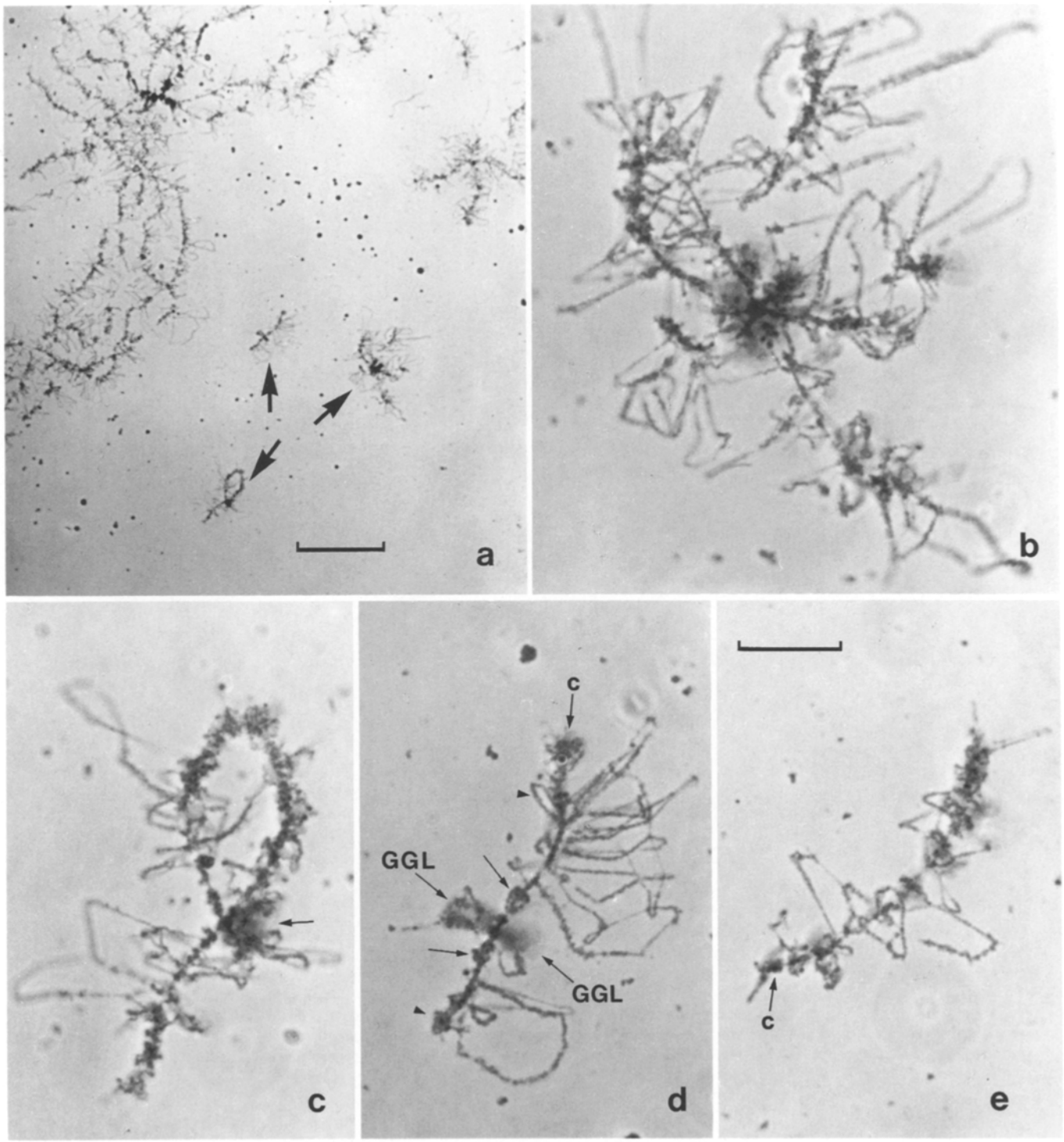



**Fig. 12a–e.** Lampbrush chromosomes from a 0.7 mm oocyte of a female *Leiopelma hochstetteri* (NMNS 29589-7) from Tapu. This individual had 12 supernumeraries plus a telocentric W chromosome. **a** Low-power view showing lampbrush autosomes (*upper right*) and supernumeraries. The supernumeraries are grouped into 3 "stars" (*arrows*) of 2, 4 and 6 elements each. The lampbrush W chromosome of this cell is not visible. Bar represents 50 μm. **b** Six-pointed lampbrush star of supernumeraries. The central core is surrounded by the Giant Granular Loops (GGLs) of the chromosomes, such as were seen in the lampbrush supernumeraries in Figure 11b, d. Other landmarks are difficult to discern. **c** Four-pointed lampbrush supernumerary star. The GGLs of the telomere ends are again evident (*arrow*) at the core of the star. One arm of the star is obscured but four lampbrush chromosome axes can be observed emerging from the core. **d** Two-pointed lampbrush star. Landmarks are clearly visible. Two GGLs (*GGL*) and a pair of dense telomeres identify the joined ends of the chromosomes. Two smaller, granular structures (*small arrows* and *arrowheads*) are symmetrically arranged on the two chromosomes, with zones of transcriptional loops between them. The distal, centromere portion (*c*) appears to have been lost from the lower chromosome. **e** Univalent lampbrush W chromosome from the same cell as **a–d.** Small granular structures are present along its length and it lacks a telomeric GGL. The centromere (*c*) is visible. Compare with the lampbrush W chromosome in Figures 10b and 11c. **b, c, d, e** are to the same scale; bar represents 10 μm

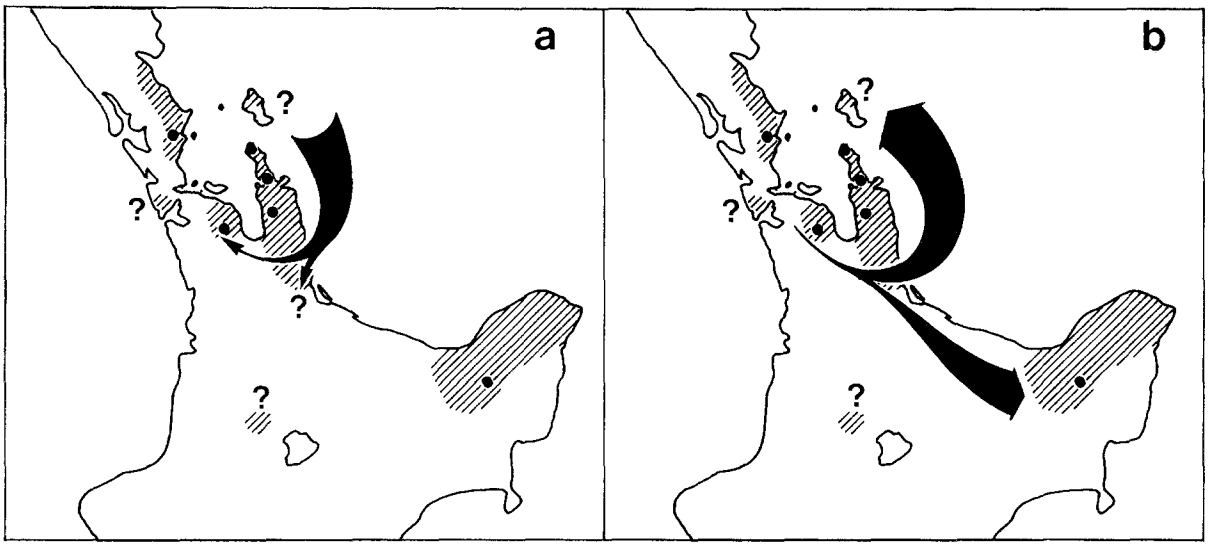



**Fig. 13.** Geographic trends in the distribution of supernumerary chromosomes and W chromosomes in *Leiopelma hochstetteri*. **a** Numbers of supernumerary *ts* chromosomes are high in the Coromandel populations, where they are presumed to have originated. The highest numbers were observed at Mt. Moehau on the northern tip of the peninsula. *Dots* indicate sample sites as portrayed in Figure 1. *Question marks* indicate regions in the range of *L. hochstetteri* from which data are lacking: Great Barrier Island to north, the Waitakere ranges to the west, the Rangitoto ranges to the south, and the southern part of the Coromandel region in the centre. **b** The incidence of metacentric W chromosomes increases along the Coromandel peninsula and eastward to the East Cape region. Unlike **a**, this map does not portray any postulated interpopulational spread of *mW* chromosomes, which appear to have arisen independently in separate populations

number of 12 *ts* chromosomes present in mitotic preparations from this frog. Telomeres of homologous lampbrush chromosomes have on occasion been seen to fuse with one another in other species (Callan 1986). Such fusions are known mainly from salamanders (eg. Callan and Lloyd 1960; Kezer et al. 1980) but have also been seen in some frogs (Giorgi and Galleni 1972). However, multivalent lampbrush stars such as seen here in *L. hochstetteri* seem never to have been seen before in any other organisms (Callan 1986 and personal communication).

All the lampbrush *ts* supernumeraries in the stars of the Tokatea female had pronounced GGLs at their telomere ends (Fig. 12b, c, d), similar to the *ts* chromosomes seen in the Mangatangi female (Fig. 11b, d). These loops were the most prominent features of the central nucleus of each star. The locations of other landmarks on the radiating arms were also largely the same (Fig. 12d). The stars themselves are additional evidence of the homology of the *ts* chromosomes and their differentiation from the univalent *tW*.

## Discussion

### *Geographic distribution of supernumerary chromosomes*

There are clear patterns to the occurrence of supernumerary chromosomes in *L. hochstetteri* (Fig. 13). The Mt. Moehau, Tapu and Tokatea populations had high numbers of supernumerary *ts* chromosomes but, of the outlying populations, only Mangatangi featured a few supernumeraries, and those were only in females. The two most distant populations, Big Omaha and Toatoa, both were without supernumaries other than the various forms of the *W* chromosomes in females. It appears as though *ts* supernumeraries are characteristic of the Coromandel populations, perhaps having arisen there, and through population exchange and the movements of animals, they may have flowed into nearby populations such as Managatangi (Fig. 13a). The *s*-type supernumeraries should exhibit more mobility in this regard than the *W*-type chromosomes as they occur in both sexes while the various *W* chromosomes are restricted to females. The current, disrupted range of *L. hochstetteri* was probably more continuous at some time and the presently disjunct populations undoubtly exchanged genes, as well as supernumerary chromosomes.

The pattern of supernumerary occurrence between Coromandel populations is not continuous. Tokatea had fewer supernumeraries, on average, than the populations on either side of it. This may be due to sampling error or may reflect the current disjunction of populations which have pursued their own evolutionary courses.

The various forms of the *W* supernumerary chromosomes also show definite patterns of geographic distribution (Fig. 13b). Metacentric *mW* chromosomes are more prevalent towards the north of the Coromandel Peninsula and towards the East Cape. Highly heterochromatic *tW* and *mW* chromosomes are characteristic of the Toatoa population while, elsewhere, these chromosomes have normal, or even reduced amounts of heterochromatin. The *mW* isochromosomes reflect the same patterns of heterochromatin as found in the *tW* chromosomes of the same populations. This indicates that, although their occurrence appears to follow geographic trends, the iso-chromosomes arose independently from different ancestral *tW* chromosomes in the different populations.

All but 2 of the 15 females from Big Omaha/Dome Valley had but a single supernumerary *tW*; the exceptions were a triploid (Green et al. 1984a) with 2 (both presumably *tWs*) and a frog examined by Morescalchi (1967) which had 12. This latter frog is an enigma. No other diploid frog from these sites had any more than the 1 *tW* supernumerary, including the other frog that Morescalchi (1967)

examined. Dr. Joan Robb, who collected the 2 frogs sent to Morescalchi has written (personal communication) that they both came from Dome Valley. However, the chromosomes of the frog with 12 supernumeraries, as illustrated by Morescalchi (1967), are exactly as I have found consistently among frogs from the Coromandel Peninsula. It seems strange that this 1 frog, 1 of the first 2 *L. hochstetteri* to be karyotyped, should depart so radically from an otherwise clear pattern.

*Heterochromatin and supernumerary chromosome origins*

Green et al. (1987) observed that *L. hochstetteri* from Dome Valley, which had few supernumerary chromosomes, had positively stained C-bands at the centromeres of the five pairs of large metacentric chromosomes whereas frogs from Tapu, which had many supernumeraries, had very little or no C-band positive heterochromatin at those positions. This appeared to be in accordance with the idea that supernumerary chromosomes may have arisen as fragments of centromeres (Jones and Rees 1982). We see now, however, that frogs from Toatoa, which like Dome Valley and Big Omaha frogs have no *ts* supernumerary chromosomes, have C-band heterochromatin distributed in the large metacentrics precisely as seen in frogs from the Coromandel Peninsula. Thus heterochromatin distribution patterns in *L. hochstetteri* are an aspect of geographic variation and are not correlated with the presence of supernumerary chromosomes.

How the supernumerary chromosomes of *L. hochstetteri* originated is therefore still unresolved. It seems likely that the *ts* chromosomes arose in *L. hochstetteri* among populations in the Coromandel region, where they are most prevalent, but considering the very different geographic patterns of distribution and genetic functions (see below) of the *s* and *W* types of supernumerary chromosomes they doubtless had separate origins, probably by different mechanisms.

There are many ideas concerning mechanisms of supernumerary chromosome origins besides centromeric fragmentation (Jones and Rees 1982). Peeters et al. (1985) have observed the genesis of supernumerary chromosomes and aneuploidy in maize, *Zea mays*, following spontaneous cell fusion in meiosis. Even if an event such as this is extremely rare in nature, it may still be sufficient to produce a supernumerary chromosome. Abnormal meiosis in *L. hochstetteri* was observed in one male by Green et al. (1987). Once formed, supernumeraries may then proliferate, which has obviously occurred with the *ts* supernumeraries of *L. hochstetteri*. Their morphology and behaviour in the lampbrush state demonstrate the homology of the *ts* chromosomes and their ability to associate into stars during oogenesis may have facilitated proliferation. Clumping may help to prevent accidental loss of univalent supernumeraries during division.

Supernumerary chromosomes may also be subject to degeneration once they have been formed. Degenerate *Y* and *W* sex chromosomes are well known and are thought to undergo loss of genetic function, accumulation of deleterious loci, and heterochromatinisation due to their limited genetic role and mandatory heterozygosity (Charlesworth 1978; Rice 1987). These are the same conditions as experienced by supernumerary chromosomes and so a mechanism such as Muller's Ratchet (Felsenstein 1974; Charlesworth 1978) may operate to turn a newly formed supernumerary into a degenerate, heterochromatic relic, as is commonly observed (Jones and Rees 1982).

Regardless of its bearing upon the origin of supernumeraries, as discussed by Green et al. (1987), the pattern of heterochromatin distribution in the large metacentric chromosomes of *L. hochstetteri* is unusual. The centromeric heterochromatin exhibits a pattern of variation that is clearly geographic. Geographic variation of heterochromatin has been seen in frogs, *Rana aurora* (Green 1985), newts, *Triturus cristatus* (Sessions 1984), and rodents, *Rattus rattus* (Yoshida and Sagai 1975), *Uromys caudimaculatus* (Baverstock et al. 1976), *Mus musculus* (Dev et al. 1976) and *Peromyscus baileyi* (Patton 1977), among other animals. Each of these cases is somewhat different but none is like *L. hochstetteri* in having the variation in centromeric heterochromatin staining intensity largely confined to a single class of morphologically distinct chromosomes.

*Quantitative effects of supernumerary chromosomes in L. hochstetteri*

Supernumerary chromosomes have been shown to have considerable effects in some organisms. They may exert influence upon meiosis (Parker et al. 1981), fertility (Hewitt et al. 1987; Suja et al. 1986) or rate of development (Harvey and Hewitt 1979). These are quantitative effects related to the numbers of supernumerary chromosomes present rather than discontinuous, or qualitative, effects ascribable to genes (Jones and Rees 1982). In most organisms, supernumerary chromosome influence upon phenotype severely limits the numbers of supernumeraries that can occur. Yet, although *L. hochstetteri* may have as many as 15, and probably more, *ts* supernumerary chromosomes in its karyotype, there are no known effects of these extra chromosomes upon its phenotype.

Green et al. (1987) reported a case of anomalous meiosis in a male frog that had 9 supernumeraries. Some or all of the telocentric chromosomes in spermatocytes of this frog either failed to synapse or else separated prematurely. At the time, this was the only existing evidence of meiosis in *L. hochstetteri* in the presence of a high number of supernumeraries. Compared against evidence of normal meiosis in a male without supernumeraries, as presented by Stephenson et al. (1972), Green et al. (1987) suggested that this may be evidence that the supernumeraries may disrupt meiosis when present in large numbers. The present evidence clearly contradicts this suggestion. None of the males examined in the present study showed the slightest disruption of normal meiosis in spermatocyte nuclei, despite having supernumerary chromosome loads ranging from 0 to 12. The supernumeraries did not pair nor associate in any way in the spermatocyte nuclei and appeared to be randomly distributed.

It is evident that the male examined by Green et al. (1987) was a rare anomaly. Its abnormal meiosis may have been due to a genetic fault but it is not possible to ascribe it to the presence of supernumeraries. The lack of appreciable effects by the *ts* chromosomes upon the phenotype of the frogs means that it is possible for them to accumulate in the high numbers seen.

*Sex chromosomes in L. hochstetteri*

Although not specifically discussed by Green et al. (1987), the data they listed suggested that there was a sex-related

pattern of supernumerary chromosome distribution among frogs from Dome Valley. The data at the time were too limited to warrant any firm assertions but the present data show that the distribution of *W*-type supernumerary chromosomes in *L. hochstetteri* is clearly related to sex. Among the 10 frogs from Toatoa, the 5 males and 5 females were clearly different (Table 1). Each female had a single supernumerary chromosome but all males were without any. This was true also among 21 frogs from Dome Valley/Big Omaha where each female, except a triploid (Green et al. 1984a) and the frog with 12 supernumeraries described by Morescalchi (1967), had a single supernumerary (Table 1). These patterns are a significant departure from randomness.

In other populations, multiple *ts* supernumerary chromosomes are present in both sexes. However, males had, on average, consistently fewer extra chromosomes of all types than did females (Table 1). The only frogs that had no extra chromosomes were all males. Furthermore, the various *W*-type supernumeraries found only in females are distinguishable from other, *s*-type supernumerary chromosomes which occur in both sexes in the Coromandel and Managatangi populations. The *mW* chromosome is easily discerned while the *tW* is larger than the *ts* chromosomes occurring in the same animal and it has a distinctive pattern of C-band heterochromatin. It is morphologically distinctive in lampbrush preparations and it does not aggregate with other supernumerary chromosomes into the lampbrush stars. The *tW* chromosome exhibited the same general lampbrush morphology in frogs from three different populations.

These data indicate conclusively that *L. hochstetteri* has a peculiar, and perhaps unique, sex-chromosome system with female heterogamety. The single supernumerary *tW* or *mW* chromosomes are in fact morphological variants of a W chromosome in an 0W female/00 male system. There is no discernable difference between male and female karyotypes except for the W chromosome and no evidence that the W chromosome is a fragment of another chromosome. Neither is there any evidence that it is a member of a multipartite $ZW_1W_2/ZZ$ system as the W does not synapse with any other chromosome in meiosis. As a univalent, it is assured of random distribution among daughter oöcyte nuclei during meiosis. This would, in turn, ensure a 1:1 sex ratio among the offspring.

In heteromorphic sex-chromosome systems, generally, it is usually the X (or Z) chromosome that is the larger karyotypic element, in keeping with the large number of genetic loci it generally contains. The Y (or W) is frequently smaller, heterochromatic, largely inert or, occasionally, lost. Degenerate X chromosomes are extremely rare and a fixed chromosomal sex-determining system that consists solely of a Y (or W) chromosome without an X (or Z) present has not been reported (Bull 1983), making *L. hochstetteri* apparently unique. Univalent sex chromosomes have been observed in the house fly, *Musca domestica,* and in the parasitic barnacle, *Peltogasterella gracilis,* but both differ fundamentally from the situation in *L. hochstetteri.*

In many populations, *M. domestica* has XY/XX sex determination with heteromorphic sex chromosomes (Boyes 1967; Milani et al. 1967). The X is larger than the Y. However, polyfactorial sex determination seems to have arisen in some populations and there is considerable variation in the occurrence of the X and Y chromosomes. XX, XY, X0, 0Y and YY males and XX, XY and X0 females have all been recorded in nature and various all-XX or all-YY strains have been produced (Milani et al. 1967; Franco et al. 1982). Although no viable 00 individuals apparently occur, clearly the sex-determining functions of these so-called X and Y chromosomes may be lost. Both the "X" and the "Y" chromosome have become highly heterochromatic and degenerate and appear to behave little differently from ordinary supernumerary chromosomes. The occasional 0Y male flies, therefore, represent a phenomenon unrelated to that responsible for the 0W females of *L. hochstetteri.*

In the barnacle, *P. gracilis,* a single univalent chromosome is associated with sex determination (Yanagimachi 1961). Females that possess this chromosome produce small eggs while females that do not possess it produce large eggs. Small eggs grow into females, large eggs grow into males. Bull (1983) interpreted this chromosomally mediated monogeny to be a form of 0W/00 sex determination but the supernumerary chromosome in *Peltogasterella* can be either present or absent in females and thus does not determine the sex of the animal possessing it (Yanagimachi 1961). Instead, the chromosome is correlated with the type of egg produced and this, in turn, is associated with the sex of the offspring. This system is therefore more apparently a form of cytoplasmic sex determination which is altogether different from the strictly chromosomal sex determination apparent in *L. hochstetteri.*

The W chromosome of *L. hochstetteri* likely contains a locus that controls the expression of sex-related genes located on other chromosomes. As there is no Z chromosome in *L. hochstetteri,* sex determination is certainly a dominant-W effect, just as it is dominant Y in humans (Bull 1983), and is not controlled by any dosage effects (i.e. recessive X) as in *Drosophila* flies or *Caenorabditis* nematodes (Baker and Belote 1987; Hodgkin 1987). A DNA sequence containing a testis-determining factor (TDF) gene has been identified on the human Y chromosome (Page et al. 1987), which otherwise has virtually no functinal genetic loci (Goodfellow et al. 1985, 1987; Weissenbach 1987). Regulation of a sex-determining gene on the *L. hochstetteri* W chromosome would be similarly controlled merely be presence or absence.

Only two, alternative, genetic sex-determination mechanisms are plausible in *L. hochstetteri* as any system involving a contribution by a Z chromosome, as listed by Page et al. (1987), can be eliminated from consideration. Firstly, the W chromosome may have an ovary-determining factor (ODF) analogous to the TDF of mammals. This ODF would trigger differentiation of the bipotential embryonic gonad of the frog to become an ovary just as TDF triggers development of a testis. No cross-hybridization of *L. hochstetteri* DNA to a TDF-containing DNA probe would be expected if this mechanism were true. The second possibility is that *L. hochstetteri* has a TDF gene on a pair of autosomes but the W chromosome carries a suppressor gene turning off its expression. This latter mechanism may have merit considering the widespread occurrence of similar DNA sequences in the sex chromosomes of both birds and mammals (Page et al. 1987; Weissenbach 1987) and perhaps other vertebrates as well.

The genetic role of the W chromosome has interesting implications concerning the metacentric iso-W-chromosomes seen in most populations of *L. hochstetteri.* These

chromosomes would have two doses of the sex-determining gene. Dosage compensation as seen in mammals would be impossible with such an arrangement. The prevalence of *mWs* over *tWs* in many populations may indicate a selective advantage for the double dose of the female-determining gene while the co-occurrence of both *tW* females and *mW* females in the same populations may be an opportunity to investigate the effects of this dosage difference.

It is unlikely that any genes other than a sex-determining factor are located on *L. hochstetteri*'s W chromosome as males would, necessarily, be null for those genes. This would impose strong selective pressure against retention of any autosomal genes on the W, or translocation of any genes onto it. In any case, the random segregation of a univalent W chromosome would easily ensure a 1:1 sex ratio without the neccessity of some mechanism (Ashley 1987) for it to synapse with, and segregate from, a Z chromosome. In light of such elegant simplicity, it may be worth asking why other organisms do not do it the same way.

*Evolution of the sex chromosome in L. hochstetteri*

The 0W/00 system of *L. hochstetteri* is a form of female heterogamety and probably originated from ZW/ZZ through loss of the Z chromosome. Chromosomal sex-determination systems are quite varied in frogs (Schmid 1983) and both male and female heterogametic systems are known. Usually, anuran sex chromosomes are cytologically indistinguishable and recognition of the sex-determination system in many species has been possible only through breeding experiments involving sex-reversed individuals (Chang and Witschi 1955; Panse 1942; Schmid 1983). Only a few cases of cytologically distinguishable, heteromorphic sex chromosomes have been reported in frogs (Schmid 1983; Green 1988). It is thus extremely interesting to find sex-chromosome heteromorphisms and male heterogamety in both *L. hochstetteri* and and its relative, *L. hamiltoni* (Green 1988), two of the most primitive of all frogs. This is additional evidence that female heterogamety is the primitive condition in frogs and that male heterogamety in diverse other lineages of anurans probably arose on multiple occasions (Green 1988).

Alternative hypotheses to loss of the Z chromosome from a primordial ZW/ZZ system in the ancestry of *L. hochstetteri* only have evidence against them. Possibly, a female-determining gene could have shifted by translocation onto a pre-existing supernumerary element or the univalent W chromosome may represent a supernumerary fragment from a primordial W in a ZW/ZZ system. But both of these mechanisms would result in some manifest heteromorphism in the autosomes, which does not occur. Neither is there homology, demonstrable through synapsis in meiosis, evident between the W chromosome, or supernumerary chromosomes, and any of the regular chromosome set.

Sex-chromosome degeneration or loss can occur under certain conditions. Y chromosomes that have degenerated by accumulating more and more deleterious mutations can be aquired in a population when mutation rate is high relative to population size, recombination between X and Y chromosomes is suppressed, and the effect of selection on individual Y chromosome mutations is negligible. Y chromosomes with fewer mutations might then be lost from one generation to the next and be unlikely to be regenerated. This model is Muller's Ratchet (Muller 1964; Felsenstein 1974; Charlesworth 1978) and is a plausible explanation for degeneration of a sex chromosome, specifically the Y (or W) chromosome. But it seems unlikely that Muller's Ratchet could also operate to eliminate X or Z chromosomes. For every mutated X there will be large numbers of normal Xs with which recombination can occur in the homogametic sex. Rice's (1987) genetic hitchhiking mechanism is likewise applicable only to Y or W chromosome reduction. A plausible model of a mechanism specific for the reduction of the Z chromosome as seen in *L. hochstetteri* is lacking.

However, differentiation in the sex chromosomes of *L. hochstetteri* does appear to conform to models proposed by Charlesworth et al. (1987) concerning relative rates of evolution of sex chromosomes and autosomes. In fact, *L. hochstetteri* may be a fine illustration of the point made by Charlesworth et al. (1987) that sex chromosomes can and should evolve at a faster rate than autosomes. There is significant interpopulational variation of heterochromatin in the autosomes of *L. hochstetteri* but far more morphological variation occurs in the W chromosomes with four, distinct C-band patterns identifiable and evidence for multiple origins for the various forms of iso-W-chromosomes specific to each population. Rapid evolution in sex chromosomes is related to the fact that they are heterozygous one-half or all of the time and occur at a lesser frequency within a population than do autosomes (Charlesworth et al. 1987). There are only three-fourths the number of X, or Z, chromosomes and one-fourth the number of Y, or W, chromosomes in a population as there are any given autosome. Specific mechanisms such as Muller's Ratchet may contribute to rapid change in the Y or W, but accelerated evolution also is true of the X and Z. Perhaps this phenomenon of rapid Z chromosome evolution may be related to the Z chromosome loss seen in *L. hochstetteri.*

*Acknowledgements.* I am grateful to the New Zealand Wildlife Service and Brian D. Bell for permission to collect and export frogs from New Zealand. Valuable advice for collecting frogs in New Zealand was provided by Drs. Ben Bell, Alison Cree and Charles Daugherty and I thank Rod Hitchmough for field assistance in the cause of catching frogs. I also thank Dr. Daugherty and his family for their hospitality. Tim Sharbel provided laboratory assistance. I am grateful to Dr. H.G. Callan for making the lampbrush preparations and instructing me in lampbrush techniques and to Dr. J.G. Gall in whose laboratory the lampbrush preparations were made. The manuscript was read and commented upon by Drs. H.G. Callan, J.P. Bogart and G. Bell. Finally, I thank Dr. Jim Kezer who first showed me *Leiopelma* chromosomes. This research was funded in part by NSERC Canada Grant UO-526 and by a McGill University Equipment Grant.

## References

Ashley T (1987) Meiotic behavior of sex chromosomes: what is normal? Chromosomes Today 9:184–195

Baker BS, Belote JH (1987) Sex determination and dosage compensation in Drosophila melanogaster. Annu Rev Genet 17:345–393

Baverstock PR, Watts CHS, Hogarth JT (1976) Heterochromatin variation in the Australian rodent Uromys caudimaculatus. Chromosoma 57:397–403

Bell BD (1982) The amphibian fauna of New Zealand. In: Newman

DG (ed) New Zealand herpetology. New Zealand Wildlife Service, Wellington, pp 27–89
Bell BD (1985) Conservation status of the endemic New Zealand frogs. In: Grigg G, Shine R, Ehrmann H (eds) Biology of Australasian frogs and reptiles. Surrey Beatty and Sons, Chipping Norton, pp 449–458
Bell BD (1986) The conservation status of New Zealand wildlife. Occ Publ NZ Wildlife Serv 12:1–103
Bogart JP (1981) Chromosome studies in Sminthillus from Cuba and Eleutherodactylus from Cuba and Puerto Rico (Anura, Leptodactylidae). Life Sci Contrib R Ontario Museum 129:1–22
Boyes JW (1967) The cytology of muscoid flies. In: Wright JW, Pal R (eds) Genetics of insect vectors of disease. Elsevier, Amsterdam, pp 371–384
Boyes JW, Van Brink JM (1967) Chromosomes of syrphidae III. Karyotypes of some species in the tribes Milesiini and Myoleptini. Chromosoma 22:417–455
Bull JJ (1983) Evolution of sex determining mechanisms. Benjamin/Cumming, Menlo Park, California
Callan HG (1986) Lampbrush chromosomes. Springer, Berlin Heidelberg New York
Callan HG, Lloyd L (1960) Lampbrush chromosomes of crested newts Triturus cristatus Laurenti. Philos Trans R Soc London Ser B 243:135–219
Callan HG, Gall JG, Berg CA (1987) The lampbrush chromosomes of Xenopus laevis: preparation, identification and distribution of 5s DNA sequences. Chromosoma 95:236–250
Chang CY, Witschi E (1955) Breeding of sex-reversed males of Xenopus laevis Daudin. Proc Soc Exp Biol Med 89:150–152
Charlesworth B (1978) Model for the evolution of Y chromosomes and dosage compensation. Proc Natl Acad Sci USA 75:5618–5622
Charlesworth B, Coyne JA, Barton NH (1987) The relative rates of evolution of sex chromosomes and autosomes. Am Nat 130:113–146
Dev VG, Miller DA, Tantravani RR, Schreck RR, Roderick TH, Erlanger BF, Miller OJ (1976) Chromosome markers in Mus musculus: differences in C-banding between the subspecies M. m. musculus and M. m. molossinus. Chromosoma 53:335–344
Duellman WE, Trueb L (1986) The biology of amphibians. McGraw-Hill, New York
Felsenstein J (1974) The evolutionary advantage of recombination. Genetics 78:737–756
Franco MG, Rubini PG, Vecchi M (1982) Sex determinants and their distribution in various populations of Musca domestica L. of western Europe. Genet Res 40:279–293
Giorgi F, Galleni L (1972) The lampbrush chromosomes of Rana esculenta L. (Amphibia-Anura). Caryologia 25:107–123
Goodfellow PN, Davies KE, Ropers HH (1985) Report of the committee on the genetic constitution of the human X and Y chromosome. Cytogenet Cell Genet 40:296–352
Goodfellow PN, Goodfellow PJ, Pym B, Banting C, Pritchard C, Darling SM (1987) Genes on the human Y chromosome. In: Hazeltine FP, McClure ME, Goldberg EH (eds) Genetic markers of sex determination. Plenum Press, New York, pp 99–111
Green DM (1985) Differentiation in amount of centromeric heterochromatin between subspecies of the red-legged frog, Rana aurora. Copeia 1985:1071–1074
Green DM (1988) Heteromorphic sex-chromosomes in the rare and primitive frog Leiopelma hamiltoni from New Zealand. J Hered 79:165–169
Green DM, Sharbel TF (1988) Comparative cytogenetics of the primitive frog Leiopelma archeyi (Amphibia, Anura). Cytogenet Cell Genet, in press
Green DM, Bogart JP, Anthony EH, Genner DL (1980) An interactive, microcomputer based karyotype analysis system for phylogenetic cytotaxonomy. Comput Biol Med 10:219–227
Green DM, Kezer J, Nussbaum RA (1984a) Triploidy in Hochstetter's frog Leiopelma hochstetteri from New Zealand. NZ J Zool 11:457–461
Green DM, Myers PZ, Reyna DL (1984b) CHROMPAC III: an improved package for micro-computer assisted analysis of karyotypes. J Hered 75:143
Green DM, Kezer J, Nussbaum RA (1987) Supernumerary chromosome variation and heterochromatin distribution in the endemic New Zealand frog, Leiopelma hochstetteri. Chromosoma 95:339–344
Harvey AW, Hewitt GM (1979) B-chromosomes slow development in a grasshopper. Heredity 42:397–401
Hewitt GM, East TM, Shaw MW (1987) Sperm dysfunction produced by B-chromosomes in the grasshopper Myrmeleotettix maculatus. Heredity 58:59–68
Hodgkin J (1987) Sex determination and dosage compensation in Caenorabditis elegans. Annu Rev Genet 21:133–154
Hsu TC (1981) Polymorphism in human acrocentric chromosomes and the silver staining method for nucleolus organizer regions. Karyogram 7:45
Jones RN, Rees H (1982) B-Chromosomes. Academic Press, New York, p 266
Kezer J, Sessions SK (1979) Chromosome variation in the plethodontid salamander. Aneides ferreus. Chromosoma 71:65–80
Kezer J, Léon PE, Sessions SK (1980) Structural differentiation of the meiotic and mitotic chromosomes of the salamander Ambystoma macrodactylum. Chromosoma 81:177–197
Levan A, Fredga D, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220
Milani R, Rubini PG, Franco MG (1967) Sex determination in the housefly. Genetica Agraria 21:385–411
Morescalchi A (1967) The karyotype of two specimens of Leiopelma hochstetteri Fitz. (Amphibia Salientia). Caryologia 21:37–46
Muller HJ (1964) The relation of recombination to mutational advance. Mutat Res 43:165–229
Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack J, McGillivray B, de la Chappelle A, Brown LG (1987) The sex-determining region of the human Y chromosome encodes a finger protein. Cell 51:1091–1104
Panse K (1942) Sur la digametie du crapaud hermaphrodite. Rev Suisse Zool 49:185–189
Parker JS, Ainsworth CC, Taylor S (1981) The B-chromosome system of Hypochoeris maculata II. B-effects on meiotic A-chromosome behaviour. Chromosoma 67:123–143
Patton JL (1977) B-chromosome systems in the pocket mouse, Perognathus baileyi: meiosis and C-band studies. Chromosoma 60:1–14
Peeters JP, Griffiths AJF, Wilkes G (1985) In vivo karyotypic modifications following spontaneous cell fusion in maize (Zea mays L.). Can J Genet Cytol 27:580–585
Rice WR (1987) Genetic hitchhiking and the evolution of reduced gene activity of the Y sex chromosome. Genetics 116:161–167
Sannomiya M (1974) Cytogenetic studies on natural populations of grasshoppers with special reference to B chromosomes. I. Gonista bicolor. Heredity 32:251–265
Schmid M (1978) Chromosome banding in amphibia I. Constitutive heterochromatin and nucleolus organizer regions in Bufo and Hyla. Chromosoma 66:361–388
Schmid M (1983) Evolution of sex chromosomes and heterogametic systems in Amphibia. Differentiation 23 (Suppl):S13–S22
Sessions SK (1984) Cytogenetics and evolution in salamanders. Ph D dissertation. University of California, Berkeley
Stephenson EM, Robinson ES, Stephenson NG (1972) Karyotype variation within the genus Leiopelma (Amphibia: Anura). Can J Genet Cytol 14:691–702
Stephenson EM, Robinson ES, Stephenson NG (1974) Inter-specific relationships of Leiopelma (Amphibia: Anura). Further karyological evidence. Experientia 30:1248–1250
Suja JA, Gosalvez J, Lopez-Fernande C, Rufas JS (1986) A cyto-

genetic analysis in Psophus stridulus (L.) (Orthoptera: Acrididae): B-chromosomes and abnormal spermatid nuclei. Genetica 70:217–224

Weissenbach J (1987) A molecular analysis of the human Y chromosome. Chromosomes Today 9:165–174

Worthy TH (1987) Osteology of Leiopelma (Amphibia: Leiopelmatidae) and descriptions of three new subfossil Leiopelma species. J R Soc NZ 17:201–251

Yanagimachi R (1961) Studies on the sexual organization of the Rhizocephala III. The mode of sex-determination in Peltogasterella. Biol Bull 120:272–283

Yoshida TH, Sagai T (1975) Variation of C-bands in the chromosomes of several subspecies of Rattus rattus. Chromosoma 50:283–300

Received March 18, 1988 / revised form May 6, 1988
Accepted by H.C. Macgregor