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C-Banding Studies on Australian Hylid Frogs: Secondary Constriction Structure and the Concept of Euchromatin Transformation

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Abstract. A C-banding and silver staining analysis of 12 species of Australian frogs of the genus *Litoria*, has shown that 6 morphologically distinct classes of secondary constrictions are present. These constrictions are distinguished by the distribution and type of C-banding chromatin and the distribution of silver staining material. Not all of these constrictions are nucleolus organizers. Groups of closely related species often share particular constrictions, although previously unencountered constrictions do occur in some species. It is argued that changes in position of nucleolar organizing constrictions is most easily explained by the amplification of latent nucleolus organizing sites. One of the more unusual features of this group of species is the shared similarity in gross chromosome morphology, contrasted to the extensive C-banding variation at secondary constriction sites. While in some of these cases chromosomal evolution has undoubtedly proceeded by the addition of heterochromatic segments, the predominant mechanism of change appears to involve the large scale transformation of euchromatin to heterochromatin.

Introduction

One of the primary features which has emerged from comparative karyotypic analyses of both vertebrates and invertebrates is that certain groups of species have established lineages in which one form of chromosomal rearrangement predominates. There are numerous examples; varanid lizards (pericentric inversion: King and King, 1975), the *Mus musculus-poschiavinus* complex (centric fusions: Gropp et al., 1972) and *Drosophila* (paracentric inversions: see White, 1973). White (1975) has argued that this phenomenon is the result of an orthoselective process producing a lineage of like changes from an array of different rearrangements, thus discounting the likelihood that these lineages were a product of the differential origin of certain rearrangements. However, King and John (1979) in a comparative study of acridoid grasshoppers have shown that in some species in which the C-band pattern is extensively modified, different

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elements of the karyotype have undergone the same type of amplification event. This suggests that a structural predisposition is involved as a contributory cause to this orthoselective process.

C-banding analyses of groups of closely related species sometimes reveal particular patterns of heterochromatin change which predominate in that group. In this connection, chromosomes of anuran amphibians are of particular interest for they are markedly conservative in respect of their chromosome number and morphology yet they show extraordinary variation in the distribution and morphology of secondary constrictions. For example, all but one species of the genus *Litoria* (Hylidae) analysed have $2n=26$ and share the same basic chromosome morphology; only *L. infrafrenata* has $2n=24$ (Menzies and Tippett, 1976; King unpublished).

Where groups of closely related species have been compared in *Bufo* (Bogart, 1972), *Cyclorana* (King et al., 1979) and *Litoria* (King unpublished), it is commonplace to find members of these groups sharing common secondary constrictions in terms of location and morphology. Additionally, cases are known where apparently "new" constrictions are present in particular species without any evidence for major karyotypic rearrangements having occurred, as is the case in *Cyclorana cryptotis* (King et al., 1979). As we shall see in this paper there are also cases where the location or appearance of secondary constrictions may vary erratically within a species. The aim of the present study is to determine the structural basis of this variation in the morphology of secondary constrictions in species of the genus *Litoria*, using a combination of both C-banding and silver staining. These techniques make it clear that there has been a marked and unusual modification in the internal organization of heterochromatin in this group of species.

Material and Methods

The localities from which specimens of the 12 species karyotyped in this study were obtained are shown in Table 1. Mitotic chromosomes were obtained from air dried intestinal epithelial preparations which were either stained with Giemsa for gross chromosome morphology, C-banded or silver stained.

1. Intestinal Epithelial Preparations. Intestinal epithelial cells were obtained using the same technique successfully applied to lizards and described in King and Rofe (1976).

2. C-Banding. Air dried intestinal epithelial preparations were placed in 0.02 N HCl for 30 min, rinsed in distilled water and dried. The slides were then placed in saturated BaOH soln. at 50° C for from 1 to 5 min, dipped briefly in 0.02 N HCl and again rinsed in distilled H_2O. The slides were then placed in $2\times$SSC at 65° C for 30 min, rinsed in distilled H_2O and stained in 10% Giemsa solution (pH 7 phosphate buffer) for 15 min. Slides were then rinsed in H_2O, dried and mounted.

3. Silver Staining. The technique employed was that described by Goodpasture and Bloom (1975) using $AgNO_3$.

Table 1. Localities of the 12 species of *Litoria* referred to in this paper

Species	2n	Number of specimens	Localities
L. chloris (Boulenger)	26	2	Warrie Ntl. Pk. Qld
L. peroni (Tschudi)	26	5	Balranald, Griffith, N.S.W.
L. pearsoni (Copland)	26	2	Canondale Rge., Qld.
L. phyllochroa (Gunther)	26	2	Bellingen, N.S.W.
L. moorei (Copland)	26	10	Chidlows, Forrestfield, Mt. Barker, Perth, W.A.
L. raniformis (Keferstein)	26	9	Devenport; Tas., Griffith, N.S.W. Millel, S.A.
L. adelaidensis (Gray)	26	3	Forrestfield, Perth, W.A.
L. lesueuri (Dumeril et Bibron)	26	8	Cairns, Qld., Gudgenby, A.C.T.
L. cooloolensis (Liem)	26	2	Cooloola Ntl. Pk. Qld.
L. olongburensis (Liem et Ingram)	26	2	Cooloola Ntl. Pk. Qld.
L. meiriana (Tyler)	26	8	Mitchell Plateau, W.A., Canon Hill, N.T.
L. infrafrenata (Gunther)	24	4	Tully, Qld., Edward R., Qld.

Results

It is clear that much higher levels of arm number and chromosome number changes have occurred in the evolution of the mammals in comparison with amphibians (Wilson et al., 1974) but we know little about other forms of chromosome reorganization within the amphibia. We do know that the considerable variability found in the expression of secondary constrictions in the anuran amphibians, suggests a much higher rate of chromosomal repatterning than is apparent from the numerical stability of the chromosome systems. A number of recent studies have utilized C and N banding to determine the internal chromosome structure of diverse anuran species (Bianchi et al., 1973; Ward, 1977; Schmid, 1978a, b). These studies reported considerable variation in the C-banding pattern and some variation in the structure of secondary constrictions, but did not concern themselves with closely related species complexes.

1. Karyotype Morphology

The chromosomal data presented in this study were selected from a broader analysis of 40 of the 55 species of *Litoria*. The twelve species chosen for detailed analysis had: (1) morphologically characteristic secondary constrictions which were representative of larger species groups, (2) major banding or morphological

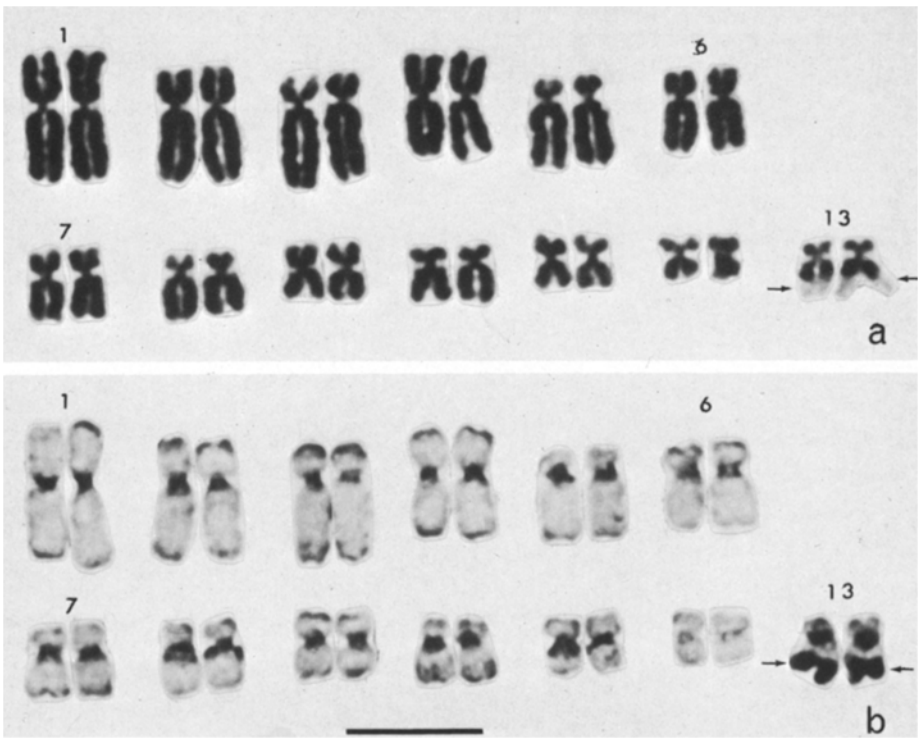

Fig. 1. a The chromosomes of *L. moorei*. Note that pair 13 has despiralized type 2 constriction without satellites (*arrowed*). In this and all other figures the bar scale is equal to 10 μm. **b** C-banded chromosomes showing procentric C bands and intense banding in the despiralized area of pair 13 (*arrowed*)

differences between pairs of species which were closely related (*L. pearsoni* and *L. phyllochroa; L. moorei* and *L. raniformis; L. cooloolensis* and *L. olongburensis*), or (3) very distinctive karyotypes (*L. meiriana, L. infrafrenata, L. adelaidensis*).

In terms of arm ratios and centromere positions the chromosome morphology of *Litoria* species is very characteristic. Pairs 1 and 4 are metacentric, pairs 2 and 6 are submetacentric and pairs 3 and 5 are acrocentric. In terms of overall size the members of the karyotype fall naturally into two clusters – pairs 1 to 6 and pairs 7 to 13. Using this combination of characters it is also possible to regularly distinguish individual members of pairs 1 to 6 although this is not the case for pairs 7 to 13. In determining the arm ratios of the chromosomes in the present study the secondary constrictions have not been included in the measurements because of marked despiralization in some cases (see Table 2).

In some five species subtle differences in centromere positions were encountered. These were attributed to pericentric inversions leading to changes in arm ratio. For example, in *L. moorei* (Fig. 1) pair 13 lacks satellites and is

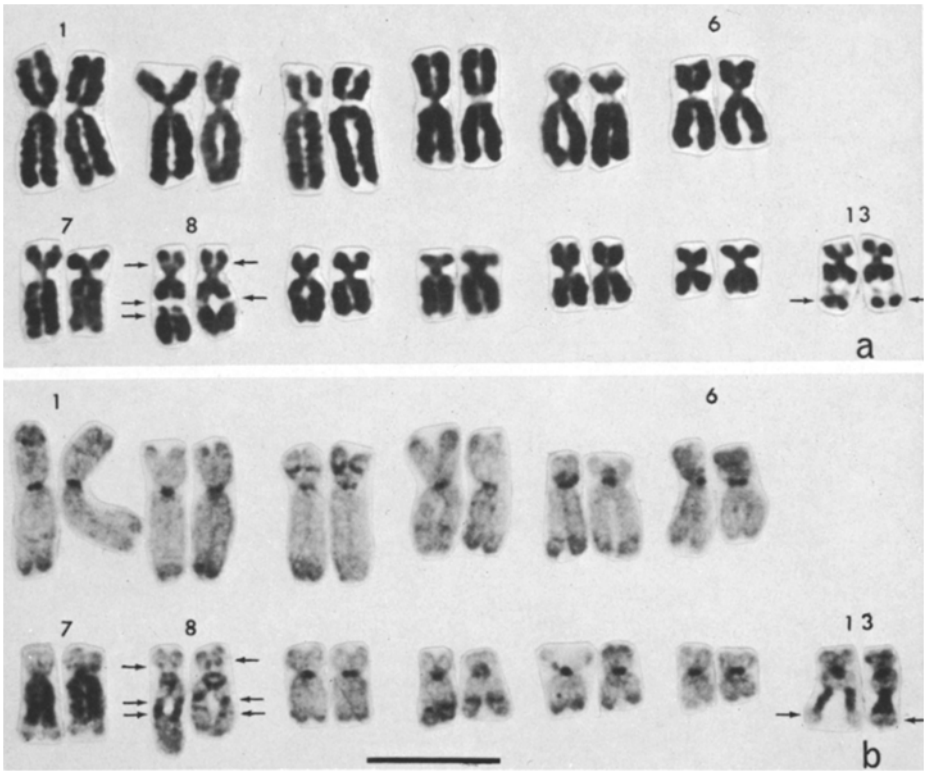

Fig. 2. a The karyotype of *L. raniformis* which has despiralized type 2 constrictions on pairs 8 (three) and 13 (one) (*arrowed*). **b** C-banded karyotype showing that the constrictions C band. A very large C block is also present in the long arm of pair 7. The satellites (*arrowed*) do not C band

more metacentric than the equivalent pair in *L. raniformis* (a sister species) which has satellites (Fig. 2). In *L. cooloolensis* and *L. olongburensis* chromosome pairs 7 to 13 are more regularly metacentric than in the other *Litoria* species (Fig. 12).

Additionally, chromosome pair 1 in *L. adelaidensis* appears to be more metacentric than in other *Litoria* species. This may either be the result of a small pericentric inversion or else stem from the presence of a small constriction in the short arm of this chromosome.

With the exception of *L. infrafrenata* (2 n = 24) all other species examined had 2n = 26. Menzies and Tippett (1976) suggested that the karyotype of *L. infrafrenata* was the product of a translocation between a pair of small and medium sized elements which reduced the chromosome number to 2n = 24. However the chromosome dimensions shown here (Table 2) offer little support for such a claim.

Table 2. Measurements of *Litoria* chromosomes given in percentage total chromosome length for the short arm (SA) and long arm (LA). The arm ratios (AR) are also shown. Asterisks designate chromosomes with constrictions

		L. infra.	*L. les.*	*L. chlor.*	*L. per.*	*L. phyll.*	*L. pears.*
	SA	4.7	5.1	6.2	5.2	5.3	5.0
1	LA	7.5	8.1	7.5	7.3	6.8	6.8
	AR	1.60	1.59	1.22	1.42	1.28	1.38
	SA	3.5	4.4	4.3	4.5	4.3	4.0
2	LA	7.2	7.9	7.7	7.8	7.0	7.2
	AR	2.07	1.82	1.80	1.75	1.64	1.77
	SA	2.6	3.2	3.3	2.8	3.8	3.4
3	LA	8.0	8.6	8.5	7.6	8.2	8.2
	AR	3.12	2.65	2.61	2.69	2.16	2.42
	SA	4.9	4.4	4.5	4.4	4.8	4.4
4	LA	5.5	6.3	5.8	6.2	6.3	6.3
	AR	1.14*	1.41	1.30	1.40	1.66	1.41
	SA	2.4	1.7	1.4	3.5	2.4	2.2
5	LA	6.5	6.5	6.8	6.0	7.6	7.2
	AR	2.73	3.88	4.80	1.71*	3.13	3.23
	SA	3.3	2.5	2.9	2.9	2.7	2.9
6	LA	6.1	5.5	5.5	5.3	5.3	5.1
	AR	1.84	2.22	1.85	1.82	1.94	1.75
	SA	2.8	2.3	1.9	3.1	2.7	2.7
7	LA	6.0	4.9	5.4	5.3	4.1	4.6
	AR	2.14	2.08	2.80	1.69	1.53	1.69
	SA	2.1	2.8	2.4	2.0	1.9	1.6
8	LA	5.5	4.5	4.4	4.2	4.6	4.4
	AR	2.60	1.59	1.84	2.13	2.38*	2.73
	SA	2.6	2.0	2.5	1.9	1.9	2.6
9	LA	4.7	2.2	3.3	3.0	3.2	3.4
	AR	1.82	1.07	1.31	1.57	1.73*	1.27*
	SA	1.6	1.7	1.5	1.5	1.7	2.2
10	LA	4.0	3.2	3.5	3.4	3.3	3.2
	AR	2.54	1.84*	2.36	2.28	2.00	1.45
	SA	2.0	1.6	1.4	1.9	2.0	2.0
11	LA	2.8	3.1	2.9	3.0	2.9	3.2
	AR	1.37	1.92	2.04*	1.59*	1.45	1.59
	SA	1.5	1.6	1.4	1.6	1.7	1.3
12	LA	2.5	2.3	2.0	2.3	2.7	2.9
	AR	1.67	1.43	1.45	1.39	1.60	2.18
	SA		1.5	1.1	1.3	1.7	1.4
13	LA		2.0	1.7	4.1	2.3	1.8
	AR		1.38	1.52	1.60	1.37	1.35

Table 2 (continued)

		L. moorei.	*L. ranif.*	*L. adel.*	*L. cool.*	*L. olong.*	*L. meir.*
	SA	5.6	4.9	6.4	5.0	4.9	5.6
1	LA	7.5	7.1	7.6	8.0	7.3	7.5
	AR	1.34	1.45	1.19*	1.61	1.48	1.34
	SA	3.9	4.1	4.1	3.7	3.8	3.8
2	LA	7.6	7.2	7.2	7.1	7.9	6.9
	AR	1.96	1.73	1.77	1.95*	2.09	1.81
	SA	3.0	3.1	3.5	2.3	2.8	2.6
3	LA	7.9	7.7	6.7	8.0	8.5	9.8
	AR	2.62	2.43	1.88	3.49*	3.00	3.78
	SA	4.2	4.7	4.2	3.9	4.7	4.9
4	LA	6.1	5.7	6.0	6.0	6.6	6.3
	AR	1.44	1.19	1.42	1.54	1.41	1.29
	SA	2.3	2.4	2.4	2.6	2.6	1.9
5	LA	6.4	6.0	6.1	7.1	6.3	6.8
	AR	2.83	2.56	2.50*	2.77	2.38	3.65
	SA	2.6	2.9	2.9	2.3	2.3	3.0
6	LA	5.3	5.1	5.4	5.0	5.8	5.1
	AR	2.06	1.75	1.83	2.20	2.52	1.72
	SA	2.5	2.3	2.8	2.7	2.6	1.4
7	LA	4.3	5.2	5.0	4.2	3.7	5.4
	AR	1.73	2.28	1.77	1.56	1.42	3.95
	SA	2.2	2.2	2.9	2.6	3.0	2.6
8	LA	4.4	4.6	3.9	3.5	3.1	3.0
	AR	1.98	2.08*	1.36	1.37	1.04	1.12
	SA	2.6	2.1	3.1	2.2	2.9	2.0
9	LA	3.3	3.8	3.1	3.7	2.9	3.6
	AR	1.24	1.76	1.00*	1.69	1.00	1.83
	SA	2.0	1.6	1.8	2.4	2.3	2.5
10	LA	3.8	3.8	2.9	3.0	2.9	3.1
	AR	1.89	2.43	1.61*	1.29	1.24	1.23
	SA	2.0	2.1	1.6	2.3	2.2	2.2
11	LA	2.9	3.1	3.3	2.8	2.5	2.0
	AR	1.42	1.49	2.11	1.23	1.14	1.07*
	SA	1.8	1.7	1.6	2.0	2.1	1.8
12	LA	2.3	2.3	2.0	2.3	2.2	2.9
	AR	1.25	1.34	1.25	1.13	1.09	1.55
	SA	1.6	1.6	1.5	1.9	1.6	1.4
13	LA	2.1	2.6	1.8	2.7	2.2	2.8
	AR	1.28*	1.64*	1.23	1.45	1.32	2.00

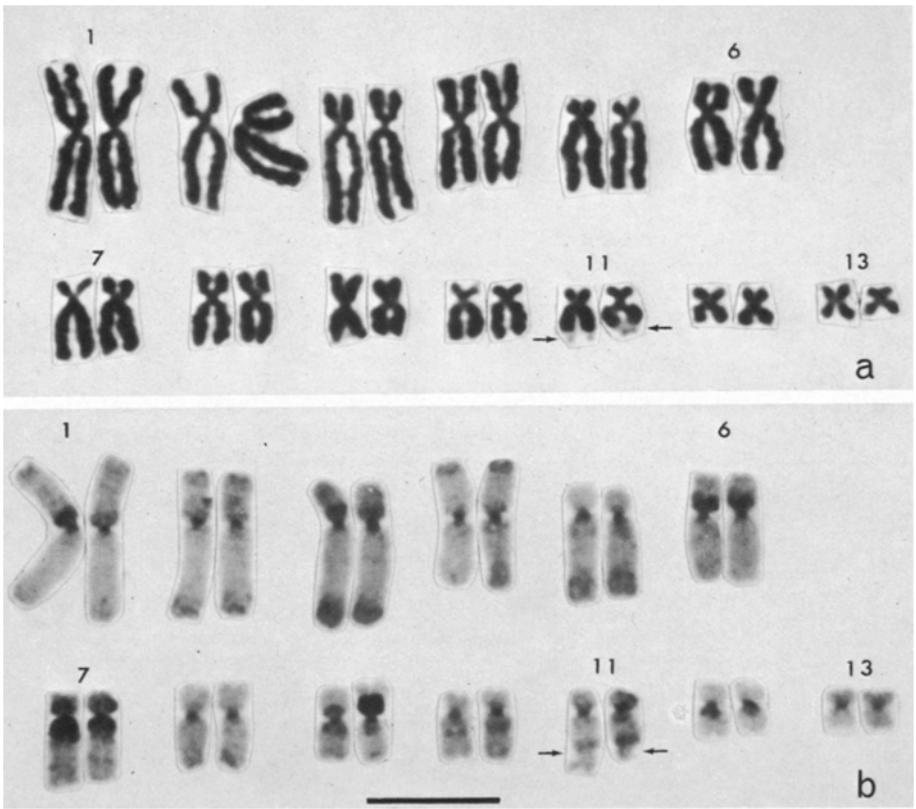

Fig. 3. **a** The chromosomes of *L. chloris* showing a type 1 constriction on the long arm of pair 11 *(arrowed)*. **b** C-banded karyotype showing procentric bands and larger blocks on pairs 6, 7 and 9, the latter of which is heteromorphic. Note the C-banding pattern of the pair 11 constriction with a band beside the despiralized area which is itself grey *(arrowed)*

2. Secondary Constriction Morphology

Many of the closely related species of *Litoria* possess a constantly expressed secondary constriction diagnostic of the group to which it belongs. These constant constrictions may show either major or minor degrees of despiralization which is directly reflected by the size of the achromatic gap. Thus, all species of the *L. aurea* complex (sensu Tyler and Davies, 1978) represented here by *L. moorei* (Fig. 1) and *L. raniformis* (Fig. 2), have a constantly expressed major despiralized constriction on pair 13. A number of large, green tree frog species, such as *L. chloris* (Fig. 3), have a constant, major despiralized terminal segment on pair 11. Members of the *L. lesueuri* complex (sensu Barker and Grigg, 1977) have a constant constriction with satellites on the long arm of pair 10 (Fig. 4) which shows only minor despiralization. Many other species have secondary constrictions unique to that species or else which are found in addition to the constant constrictions mentioned above; these too may show either major

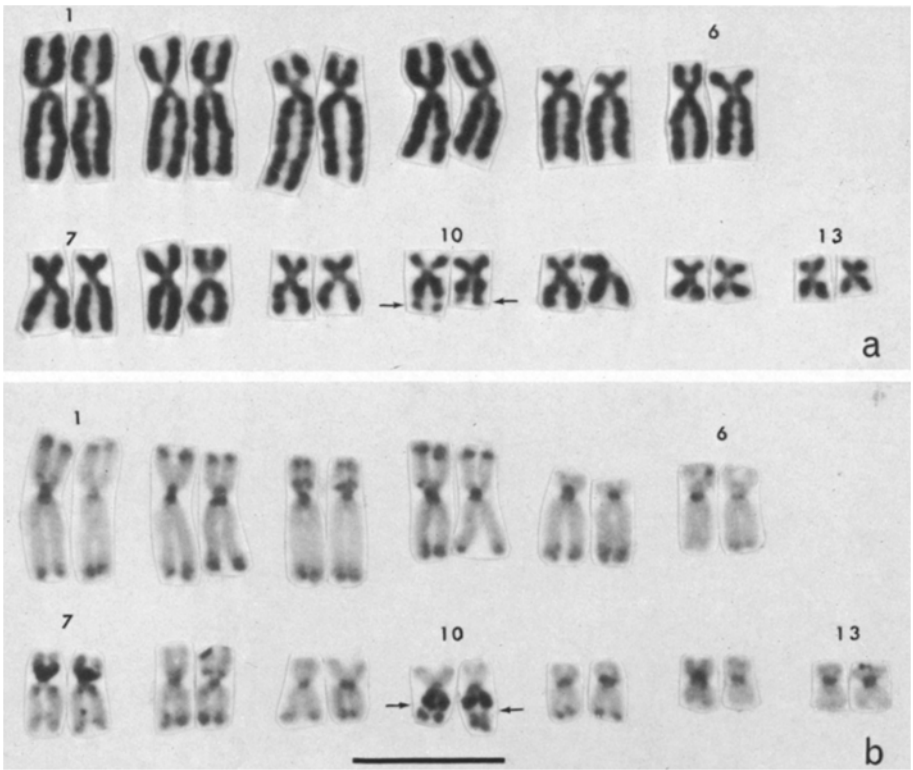

Fig. 4. **a** The karyotype of *L. lesueuri* which has a type 4 constriction on the long arm of pair 10 (*arrowed*). **b** The C-banded chromosomes showing procentric bands and grey terminal bands. Pair 7 has a large C block and the pair 10 constriction has C blocks associated with it

or minor patterns of despiralization. Some species have variable constrictions which are expressed in some, but not all, cells and vary both within and between individuals. These never show major despiralization.

The following description of the classes of secondary constrictions encountered is based on a combination of conventional staining, C-banding and silver staining.

Type 1. These constrictions are always expressed and may be terminal or subterminal, i.e., with or without satellite arms. They appear as a despiralized area of heterochromatin, often with a "spun out" appearance, vary in length between species and are frequently heterozygous for gap size between homologues. When C-banded the despiralized area has a "grey" appearance. If the constriction is subterminal, i.e., satellites are present, a dark C-band occurs on the edge of the satellite adjoining the constriction (Fig. 5). If the constriction or despiralized area is terminal then a dark C-band occurs on the region of the main arm adjoining the constriction (Fig. 5). This type is seen in *L. chloris* (Fig. 3), *L. peroni* (Fig. 6) and *L. pearsoni* (Fig. 7). Both of these categories

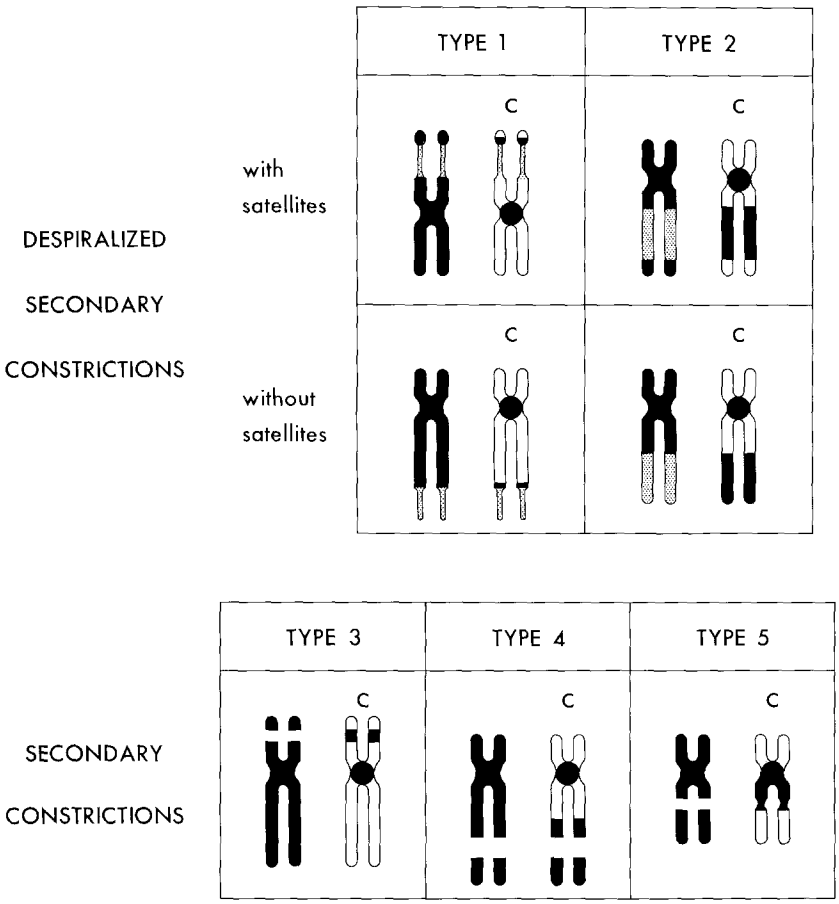

Fig. 5. A diagrammatic representation of the types of secondary constrictions seen in *Litoria* species. Giemsa stained chromosomes are shown on the left and C-banded chromosomes on the right

of despiralized constrictions silver stain and are presumed to be nucleolar organizers.

Type 2. These constant constrictions may occur terminally, subterminally or interstitially and have only been observed in members of the *L. aurea* complex (see *L. moorei* pair 13, Fig. 1, and *L. raniformis* pairs 8 and 13, Fig. 2). These constrictions have a large and often variable achromatic gap, which in some cases may be heteromorphic in size between homologues. When C-banded the gap itself stains darkly (Fig. 5). In the extreme forms of heteromorphism there may be over 3 times the amount of C-banded material in one homologue when compared to the other (Fig. 8). This dimorphism in the size of the secondary constriction appears to be analogus to the situation found in *Rana blairi* by Ward (1977) and in *Plethodon cinereas* by MacGregor et al. (1977) who proposed that this was a direct reflection of the number of ribosomal cistrons

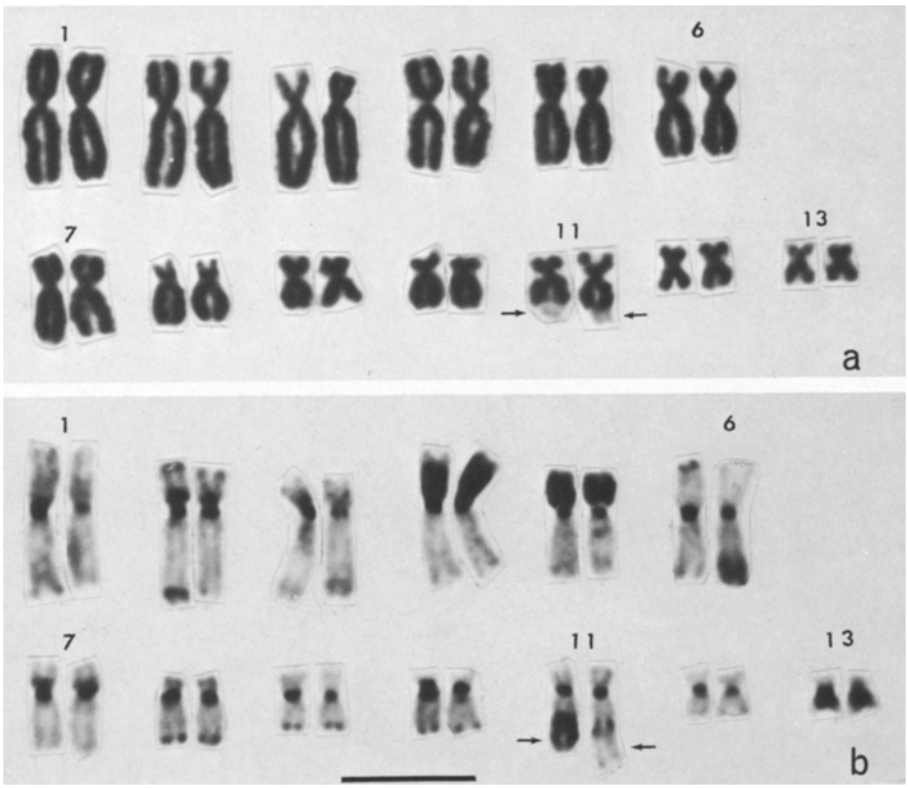

Fig. 6. a The chromosomes of *L. peroni*. Note the type 1 constriction on the long arm of pair 11 (*arrowed*). **b** This C-banded karyotype shows large procentric C bands and C blocks on pairs 4 and 5 (heterozygous). Note the C bands associated with the pair 11 constriction (*arrowed*)

present on the homologues. The achromatic gap silver stains and is regarded as a nucleolor organizer (Fig. 9). There is a direct correspondence between the area which C-bands and that which silver stains (Fig. 9).

Type 3. These variable constrictions are the most common class encountered. They vary in their expression between chromosomes, cells and individuals. Their position coincides with that of a small C-band (Figs. 5 and 10). They are presumably analogous to the cold induced despiralized constrictions of Rudak and Callan (1976). These constrictions lack any silver staining reaction and are not regarded as nucleolar organizing sites.

Type 4. These constant constrictions may be terminal, subterminal or interstitial. They have a very prominent achromatic gap which is uniformly expressed. When C-banded the constriction itself is free of C-banding, but is surrounded by very large C-banded blocks (Fig. 5). This type is found in the *L. lesueuri* complex on pair 10 (Fig. 4) and even when in a terminal position, as in *L. infrafrenata* (Fig. 11), possesses a major C-banded block. These constrictions silver stain and are regarded as nucleolar organizers. They appear to be analo-

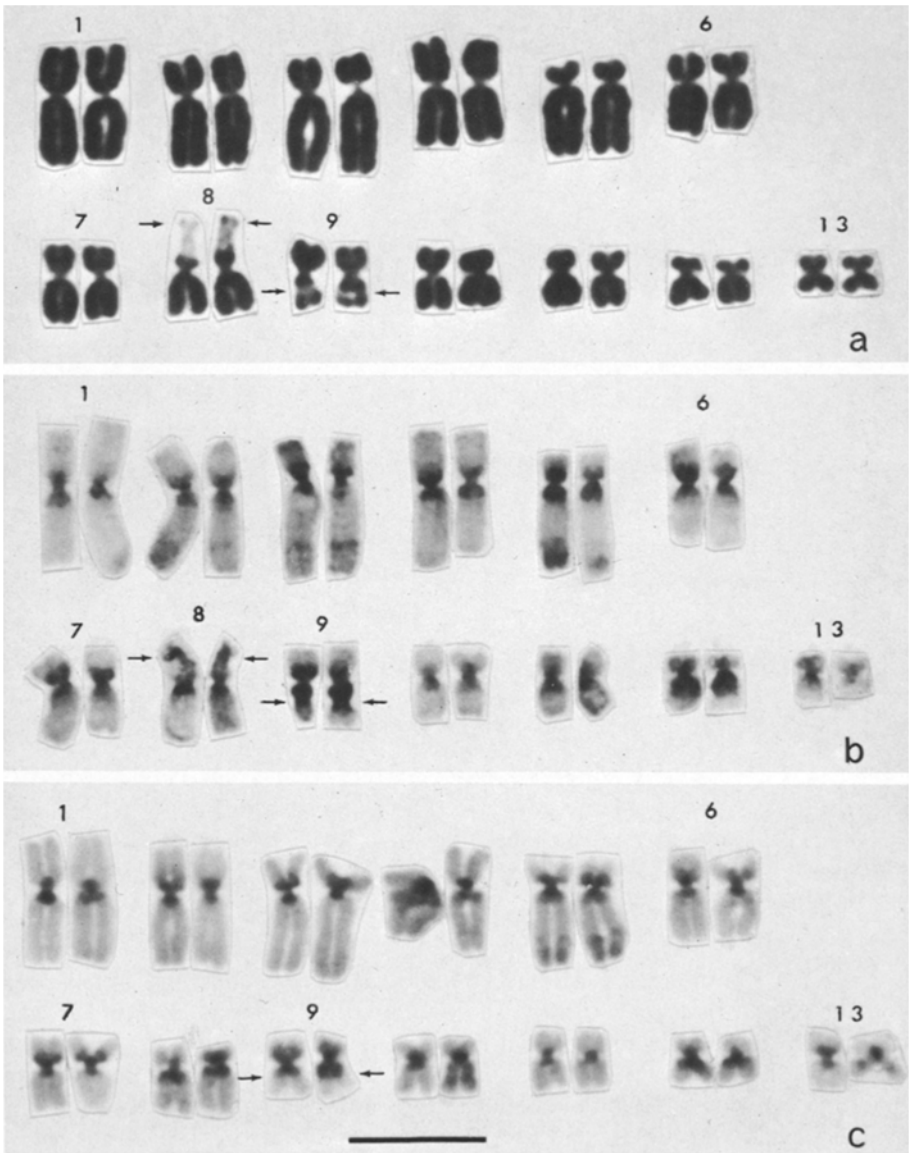

Fig. 7. **a** The chromosomes of *L. pearsoni*. Note the type 1 constriction on pair 8 (*arrowed*) and type 5 constriction on pair 9 (*arrowed*). **b** The C-banded karyotype of *L. pearsoni* showing procentric bands and terminal grey bands. Note the C-banding patterns of pairs 8 and 9. **c** The C-banded karyotype of *L. phyllochroa* which has a type 4 constriction on pair 9 (*arrowed*)

gous to similar regions found in species of Bufonidae, Hylidae, Ranidae, Microhylidae and Rhacophoridae by Schmid (1978a, b).

Type 5. This constant constriction was only observed in one species, *L. pearsoni* (pair 9, Fig. 7b), in which it was always expressed. When C-banded

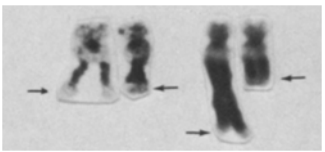

Fig. 8. C-banded type 2 constrictions of pair 13 in two specimens of *L. raniformis*. The pair on the left is homomorphic for the constriction whilst that on the right is markedly heteromorphic. Note that the amount of C-banded material is directly proportional to the size of the achromatic gap

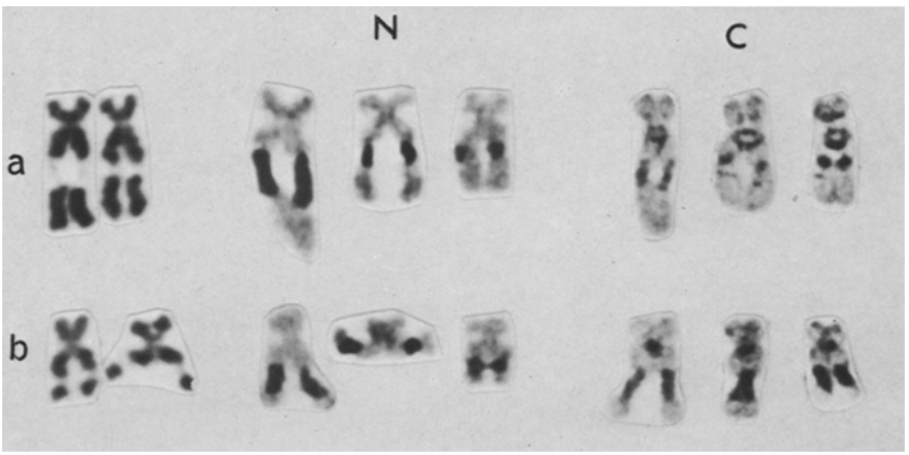

Fig. 9. a From left to right, Giemsa stained (2), silver stained (3) and C-banded (3) pair 8 chromosomes with type 2 constrictions from *L. raniformis*. **b** Similar groupings of pair 13 chromosomes from *L. raniformis* showing type 2 constrictions. Note that in these preparations the entire achromatic gap stains with the silver and C-banding techniques. The amount of stained material varies with the size of the achromatic gap, and in this case the variation is between cells on the one slide

Table 3. A summary of the staining characteristics of the five constriction types

Constriction		C banding		Silver banding within constr.
		within constr.	around constr.	
Constant	Type 1	+ grey	+ black	+
	Type 2	+ black	–	+
	Type 4	–	+ black	+
	Type 5	+ black	+ black	?
Variable	Type 3	+ black	–	–

the achromatic gap, as well as the adjoining area, C-bands (Figs. 5 and 7). Since silver staining has not been carried out on this species it is not known whether this type is involved in organizing a nucleolus.

We may therefore summarise the known situation in *Litoria* as in Table 3. This classification of secondary constrictions highlights two discrepancies with the published literature. First, the karyotype of *L. phyllochroa*, which is described by Stephenson and Stephenson (1970) as having a despiralized constriction

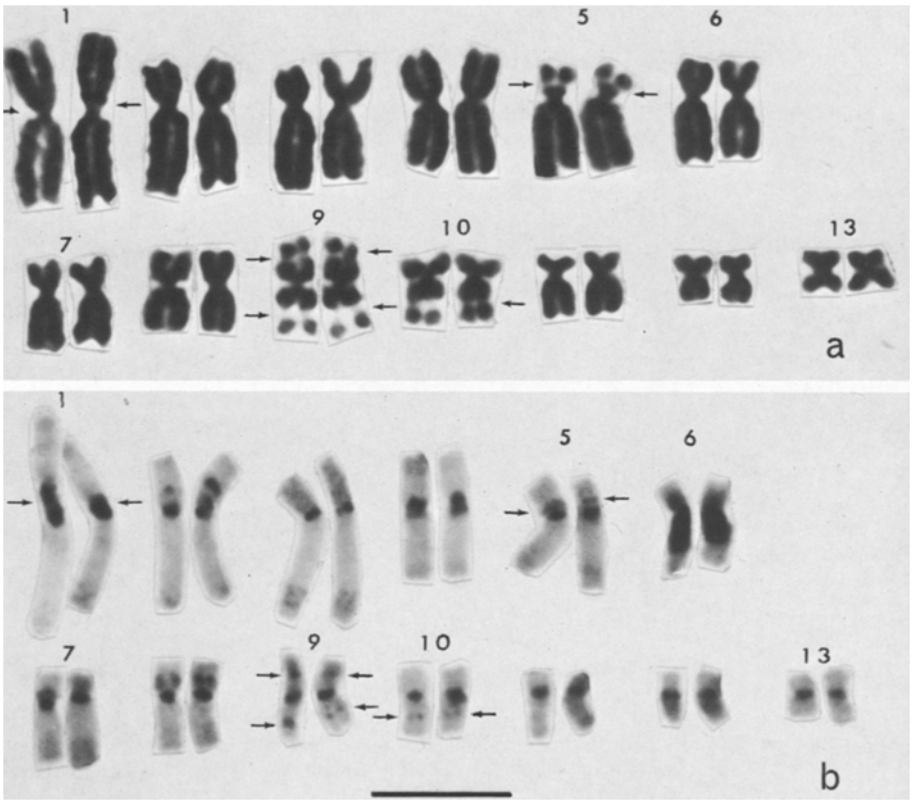

Fig. 10. **a** The chromosomes of *L. adelaidensis*. Note the type 3 constrictions on pairs 1, 5, 9 (two) and 10 (*arrowed*). **b** The C-banded karyotype of this species has a large C block on pair 6 and small C bands corresponding to the sites of type 3 constrictions (*arrowed*)

on chromosome pair 11, does not correspond to the karyotype of *L. phyllochroa* reported here (type 4 constriction pair 9) or that of its sister species *L. pearsoni* (type 1 constriction pair 8, type 5 constriction pair 9). The karyotypes shown by Stephenson and Stephenson are in fact very similar to those found in *L. caerulea, L. chloris* and *L. gracilenta* (type 1 pair 10; King unpublished) and may be the result of a misidentified specimen.

Second, Schmid (1978a) in his analysis of *Hyla* and *Bufo* species found that secondary constrictions which had C-bands within the constriction itself did not silver stain and were consequently not nucleolar organizer regions. It is quite clear from the present study that this is not necessarily the case since type 2 despiralized constrictions both silver stain and C-band within the large achromatic gap (Fig. 9). Two possible explanations for Schmid's findings are that (1) the resolution of his C-banding preparations was not adequate to exactly locate the position of the constrictions, or (2) the C-banded constrictions he encountered were of the non constant type 3.

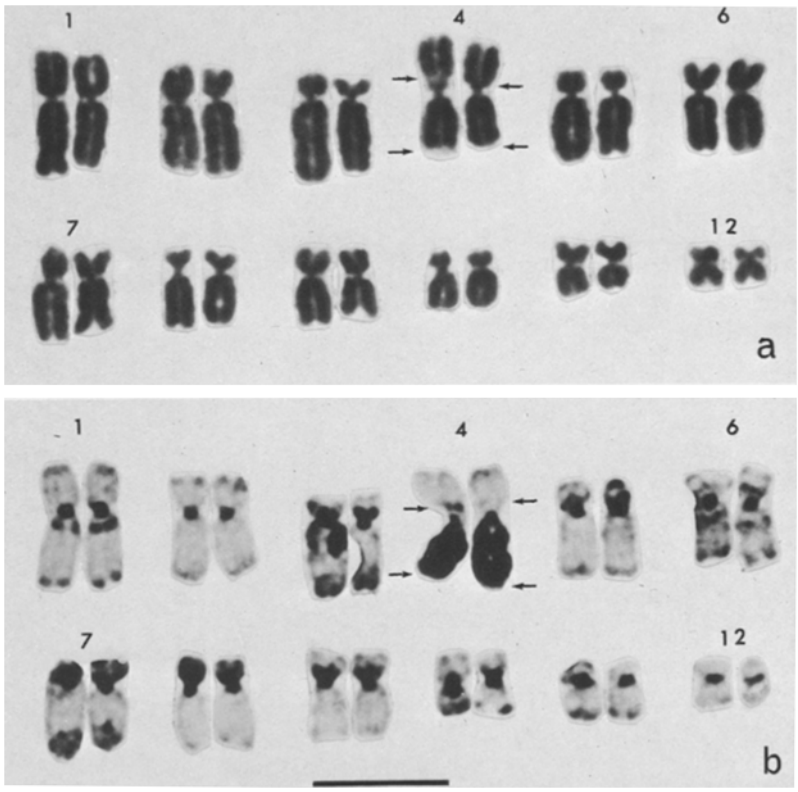

Fig. 11. a The chromosomes of *L. infrafrenata* (2n=24). A type 3 constriction is present in the short arm of pair 4 and a type 4 constriction is present on the telomeric end of the long arm of this chromosome (*arrowed*). **b** The C-banded karyotype has a whole arm C block on the long arm of pair 4 (associated with the type 4 constriction) and a small C band on the short arm associated with the type 3 constriction. Large C blocks are also present in pairs 7 and 8

3. Heterochromatin Distribution

Although the 2n=26 karyotypes of *Litoria* species are remarkably uniform in their morphology, the considerable variation in the structure of the secondary constriction implies extensive heterochromatic reorganization as shown by C-banding.

When examining the C-banding patterns between species the most striking feature is that no two of them share the same pattern, a point summarized in Figure 12. In addition to C-bands associated with secondary constrictions there are four arbitrary classes of C-heterochromatin. These are (1) procentric bands, (2) interstitial bands, (3) terminal grey bands, and (4) major C-blocks which occupy most, if not all, of a chromosome arm.

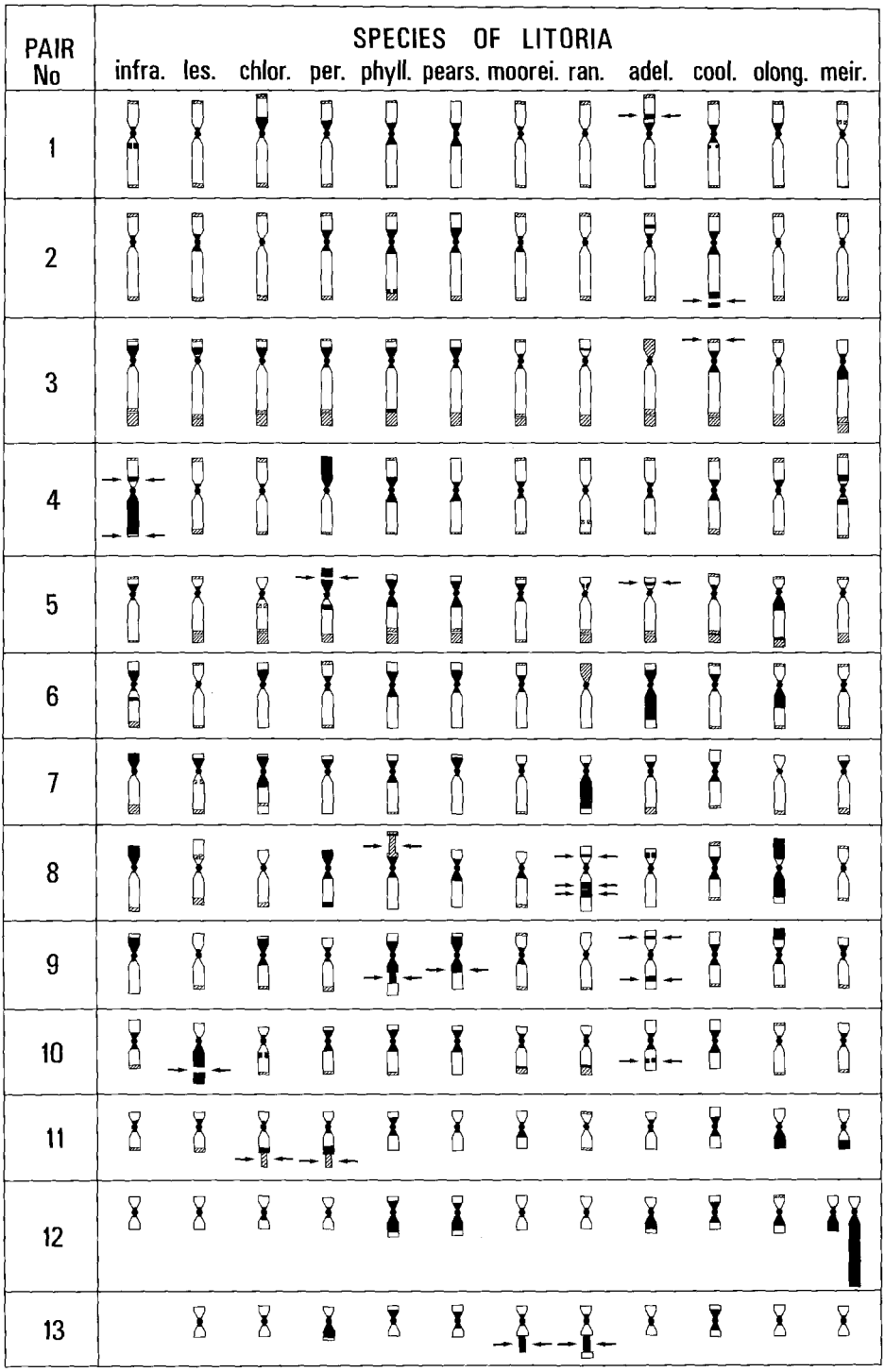

Fig. 12. A diagramatic representation of haploid C-banded karyotypes of the 12 species of *Litoria*. This figure emphasizes the marked differences in C band pattern despite the similarity in gross chromosome morphology

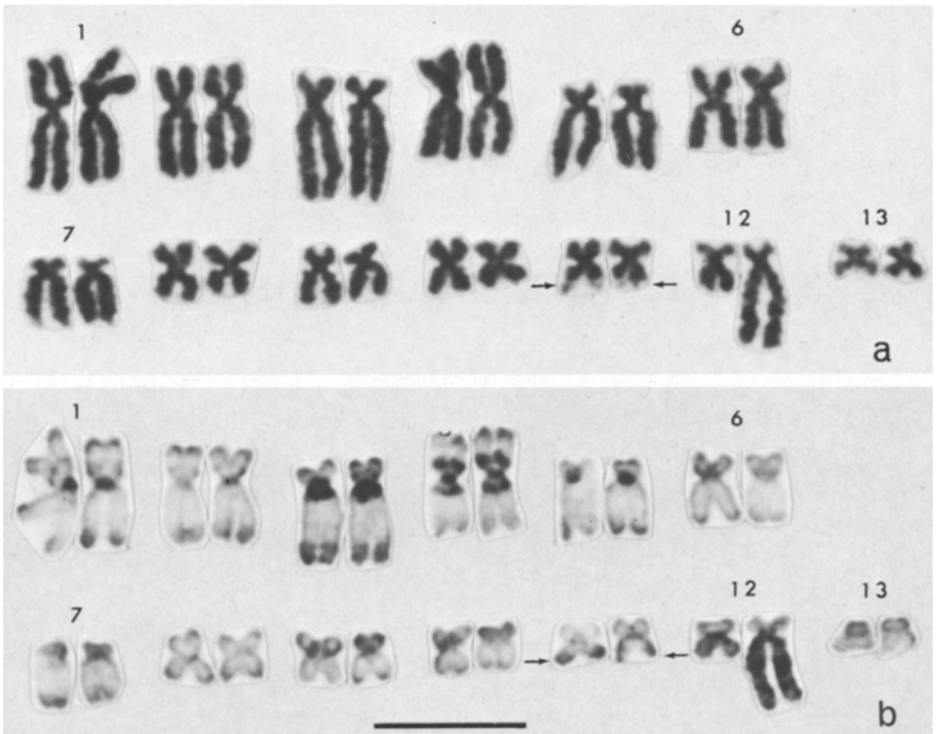

Fig. 13. a The chromosomes of *L. meiriana*. Note the heteromorphism for a very large heterochromatic block on chromosome pair 12. A type 1 constriction is present on pair 11 *(arrowed)*. **b** The C-banded karyotype shows that the pair 12 block is completely C-banded and appears to be additional material

a) Procentric Bands

There is remarkable variation in the quantity and distribution of these C-bands both between chromosomes and between karyotypes in *Litoria*. Species such as *L. raniformis* and *L. lesueuri* have uniformly small procentric bands, whereas *L. phyllochroa, L. pearsoni, L. cooloolensis* and *L. moorei* have relatively large bands extending from the centromere into both arms (Fig. 12). Other species, such as *L. peroni, L. chloris* and *L. infrafrenata*, have small procentric C-bands in some chromosomes and large bands in other (Fig. 12). Similarly, species such as *L. raniformis* and *L. moorei* have procentric bands uniform in terms of the area occupied in each chromosome arm whereas *L. chloris* (pairs 3 and 6), *L. phyllochroa* (pair 3), *L. infrafrenata* (pairs 3, 5, 6, 7, 8 and 9) and *L. adelaidensis* (pairs 1, 6, 7, and 12) have large procentric blocks which extend preferentially into one arm rather than the other (Fig. 12). In these cases, no change in the relative length of the arm has accompanied this disproportionate accumulation of C-banded material and pairs 1–6 retain their relative karyomorphology. In *L. meiriani*, on the other hand, the large procentric block on pair 3 has produced a noticeable increase in chromosome arm length, and

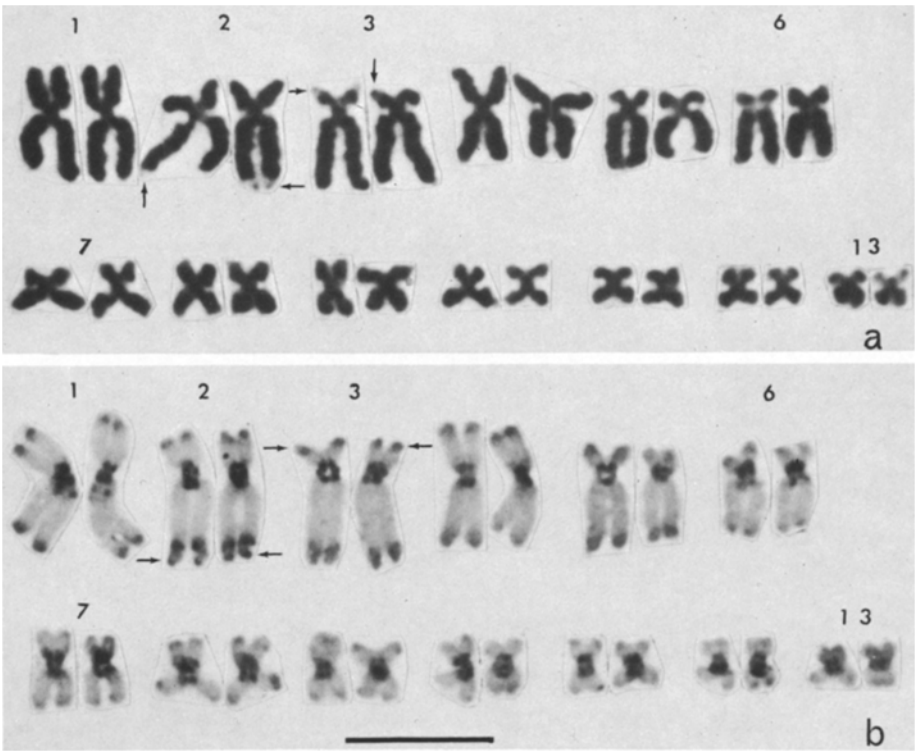

Fig. 14. **a** The chromosomes of *L. cooloolensis* showing type 4 constrictions on pair 2 and type 3 constrictions on pair 3 (*arrowed*). **b** The C-banded karyotype of *L. cooloolensis*

must therefore result from the addition of C-banded material (Fig. 13). The same is true of the procentric blocks on pair 1 in *L. chloris* and pair 12 in *L. pearsoni* and *L. phyllochroa*.

b) Interstitial Bands

Relatively few interstitial C-bands were encountered and, when present, they occurred only as fine bands. They were found in *L. raniformis* (pair 3), *L. peroni* (pair 5), *L. lesueuri* (pairs 3, 7, 10) and *L. infrafrenata* (pairs 4 and 6). By contrast, *L. adelaidensis* has interstitial C-bands on pairs 1, 2, 5, 8, 9 and 10. These often appear as type 3 constrictions in conventionally stained preparations. In *L. infrafrenata* a band on the short arm of pair 4 is also expressed as a type 3 constriction. Two larger interstitial C-bands are present in *L. meiriana* (pair 4) and are associated with an increase in arm length (Fig. 12).

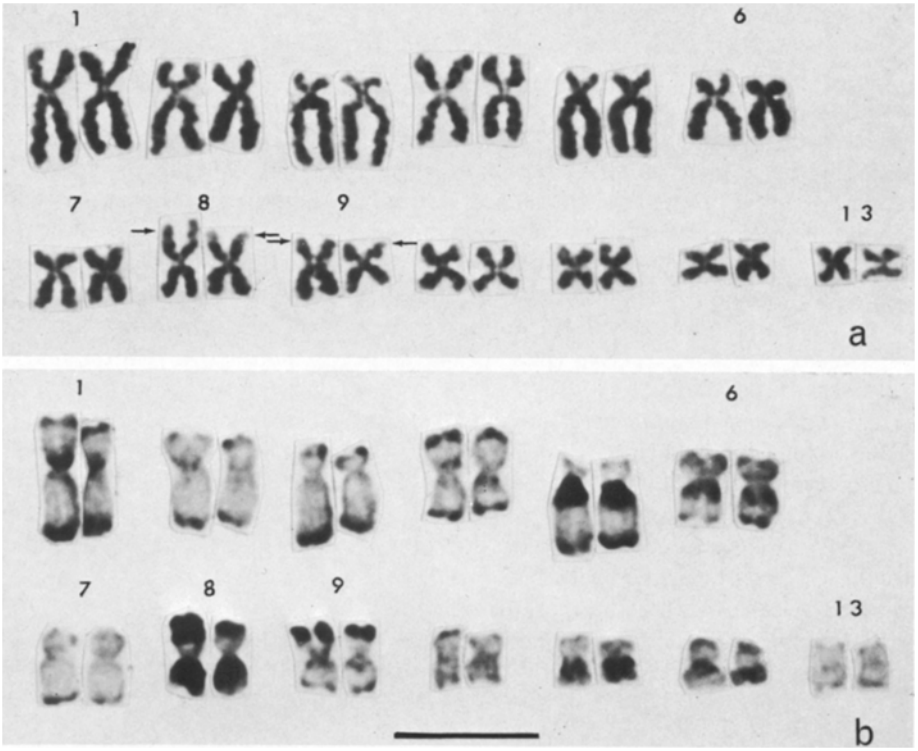

Fig. 15. **a** The chromosomes of *L. olongburensis* with unusual despiralized areas on the short arms of pairs 8 and 9 (*arrowed*). **b** The C-banded karyotype shows large C-blocks on pairs 5, 8 (two) and 9

c) Terminal Grey Bands

Lighter grey C-bands are present in most chromosomes of all species and appear in the telomeric regions. The expression of these bands is often variable and is to some degree dependent on length of exposure to barium hydroxide. Yet in a number of cases they are remarkably consistent, e.g. a large double barred grey C-band is present on the long arm of pair 3 in all species (Fig. 12). A similar band is present on the long arm of pair 5 in most species (Fig. 12). Whole short arms appear grey in *L. adelaidensis* (pair 3) and *L. raniformis* (pair 6). Grey C-bands are also present in despiralized Type 1 secondary constrictions (Fig. 5).

d) Major C-Blocks

One of the most striking features of this study is the high incidence of large darkly stained C-band blocks which often occupy entire chromosome arms. On some occasions these are associated with type 4 or 5 secondary constrictions.

These blocks are expressed in two forms:

(1) In *Litoria meiriana* a very large additional and polymorphic segment is present on pair 12. This large C-block was seen only in the heterozygous condition in which it increases the size of the pair 12 to that of pair 3 (Fig. 13). The situation appears to be polymorphic since both heterozygotes and homozygotes for the absence of the block have been observed naturally in the same populations and are not sex correlated. Moreover, heterozygotes occur in two populations situated approximately 1000 km apart.

(2) The most common form of C-block occupies either a large proportion or else the whole of a chromosome arm and does not appear to have modified the external chromosome dimensions. In species such as *L. lesueuri* (pair 10), *L. pearsoni* (pair 9), *L. peroni* (pair 5) and *L. infrafrenata* (pair 4) these major C-Blocks are associated with Type 4 or 5 secondary constrictions (Fig. 12). By contrast, in *L. adelaidensis* (pair 6), *L. peroni* (pair 4), *L. infrafrenata* (pair 7) and most spectacularly *L. raniformis* (pair 7), these blocks are not associated with constrictions (Fig. 12). Moreover the dimensions of the chromosomes remain unchanged compared to those of sister species which lack such blocks: compare pair 7 in *L. raniformis* with that of *L. moorei* (Fig. 1 and 2). A similar situation is present in the species *L. cooloolensis* and *L. olongburensis* (Figs. 14 and 15) the latter of which has half arm blocks in pairs 6, 8 and 9. *L. cooloolensis* lacks these blocks (Fig. 14). Small blocks occupying from 1/3 to 1/2 chromosome arms without obviously changing chromosome morphology are also present in *L. lesueuri* (pair 7), *L. chloris* (pair 7) and *L. phyllochroa* (pair 12) (Fig. 12).

Discussion

1. Secondary Constriction Structure

A series of studies on the products and probable functions of secondary constrictions has been made on a number of amphibian species (MacGregor and Kezer, 1973; Hutchison and Pardue, 1975; MacGregor and Mizumo, 1976; MacGregor et al., 1977; Nardi et al., 1977). However, there is a sizeable gap in our knowledge between the molecular RNA/DNA hybridization studies carried out by these workers and our basic understanding of the structure of secondary constrictions at the chromosome level. This stems largely from the fact that in past studies structural classes of secondary constrictions have not been adequately defined, nor has the distribution of heterochromatin in relation to these constrictions been described. It is now clear that C-heterochromatin plays an integral role in the structure of these organelles, and provides a basis for subdividing the constrictions in *Litoria* into five structural classes.

While some of these secondary constrictions are undoubtedly nucleolar organizer sites, this can only be confidently determined by pachytene analysis. Many authors have, however, assumed that the silver staining technique applied to c-mitotic plates (Goodpasture and Bloom, 1975) adequately defines such sites. While this may be the case in some mammals it is worth noting that

in the newt *Triturus cristatus carnifex*, Varley and Morgan (1978) have demonstrated that particular chromosome loops, which are clearly not nucleolar organizers, gave a positive reaction with the silver staining technique. These authors also found that in addition to the known nucleolar organizer regions in *Xenopus laevis* certain other sites also band with the silver technique. Clearly, the silver staining technique when applied to amphibians is not completely specific to nucleolar organizer regions though it does stain them. Paralleling these observations, Nardi et al. (1977) report that in *Triturus vulgaris meridionalis* a series of sites in addition to the known nucleolar organizer hybridized to both 18 S and 28 S ribosomal RNA. There does, however, seem to be little doubt that the "major" constrictions of anurans which silver stain and also have an associated C-banding structure are in fact nucleolar organizers.

Thus, in the *Litoria* species examined here, the constantly expressed types 1, 2 and 4 constrictions are all regarded as nucleolar organizer regions. The type 3 constriction does not band and is not regarded as a nucleolar organizer site. Unfortunately, it is not known whether the type 5 constriction has an organizer function although it appears likely when its similarity to the type 4 constriction is considered.

A major problem associated with our understanding of the evolution of secondary constrictions is their apparently erratic distribution. Whilst the external karyotype morphology in most *Litoria* species remains relatively constant in terms of chromosome size and centromere position, the localization of major secondary constrictions can vary markedly between species (Fig. 12).

There are at least four possible mechanisms which could produce such a pattern of variation, namely:

(a) That multiple cryptic structural rearrangements (minute three-break insertions or small inversions) have moved the secondary constrictions throughout the karyotype. However, this cannot account for the wholesale transposition of major despiralized constrictions because it would be expected to lead to very obvious alterations in karyomorphology. The possibility remains that the transposition of type 3 constrictions by three break insertions might provide a basis for the amplification of this type of constriction so converting it to a major despiralized constriction (Type 1 or 2). While insertions of this type are certainly known to occur (Rothfels and Freeman, 1966) it is unlikely that they are sufficiently frequent to account for the variability observed in the present case.

(b) Amplification of 18 S and 28 S rDNA, which is known to occur in amphibian oogenesis where it procedures a series of extra chromosomal nucleoli in the mature oocyte, has been proposed by Nardi et al. (1977) and Schmid (1978b) as a possible mechanism leading to nucleolar organizer site changes. These authors argue that such extra chromosomal rDNA molecules may become reintegrated at various additional sites on the chromosomes thus forming additional nucleolus organizers. However, as Schmid points out, there is no experimental evidence for this hypothesis.

(c) Nardi et al. (1977) also suggest that non homologous exchange events could change the chromosomal location of the 18 S+28 S rDNA genes. Whilst this is a possibility one might expect to see changes in chromosome arm lengths

accompanying such non homologous exchange events. In *Litoria* there is no evidence that changes of this nature have occurred.

(d) The most reasonable hypothesis suggests that there are a series of latent nucleolar organizer sites throughout a karyotype and, that during the evolution of a species, particular sites take over the primary nucleolar function. Such a mechanism has been clearly recorded in the polytene nuclei of hybrids between *Drosophila mulleri* and *Drosophila arizonensis* (Bicudo and Richardson, 1977). These authors argued that a latent organizer exists in the microchromosomes of *arizonensis*. This is normally repressed but becomes activated in polytene cells of male hybrids.

It is probable that subsequent to, or during the course of this functional amplification of a site, there are a series of internal structural modifications involving the production and redistribution of heterochromatin. This reorganization of heterochromatin may produce the characteristic secondary constriction types we see in *Litoria*. Blocks of heterochromatin may be simply associated with these major secondary constrictions to prevent crossing over in them. To produce this substantial internal reorganization of the karyotype without changing the karyotypic dimensions, requires a mechanism for transforming regions of non C-banding euchromatin into major C-banding blocks. This process of euchromatin transformation will be more fully discussed in the following section.

2. Modes of Heterochromatin Evolution in Litoria

When examining the chromosome morphology of *Litoria* species and many other anurans, one is struck by an apparent uniformity of chromosome number, relative chromosome size and centromere position (Morescalchi, 1973; Bogart, 1973). This feature has been responsible for the widely expressed attitude that frogs are chromosomally conservative. Wilson et al., (1974) have gone so far as to argue that anurans have approximately twenty times fewer chromosomal rearrangements (translocations, inversions) than mammal species. It is undoubtedly true that many species of anurans are chromosomally conservative in terms of lack of gross chromosome change. There are, however, certain groups of South American hylid species (Bogart, 1973) and Eleuthrodactylid species (Bogart, 1973; Deweese, 1975) which do have relatively high levels of gross chromosome rearrangement involving fissions, fusions and inversions. If the karyotypic end point in an evolutionary series is a completely biarmed karyotype which cannot undergo successful translocation, fissions or inversions, then such a karyotype will necessarily appear in gross terms to be evolutionarily stable. However, in Anurans this endpoint is evidently more apparent than real since it does not preclude extensive changes in the amount of heterochromatin. Thus, it is clear from C-banding studies on *Litoria* and on *Hyla, Bufo* and *Rana* species (Schmid, 1978a and b) that chromosomal reorganization has indeed proceeded largely by internal adjustments in the amount and location of heterochromatin without any accompanying major structural rearrangements. Thus in *Litoria*, no two species are karyotypically identical in terms of their C-banding pattern.

Of the major classes of C-banding material observed in *Litoria* (dark procentric C-bands, interstitial C-bands, telomeric light grey C-bands and major dark C-banding blocks) it is the procentric C-bands and major blocks which give us an insight into the mode of heterochromatin evolution in these amphibians. There are two primary processes occurring; addition of heterochromatin, and transformation of euchromatin.

a) Addition of Heterochromatin

In certain instances blocks of darkly C-banded material appear to have been added to particular chromosomes. The best example is seen in *Litoria meiriana* where a polymorphism for a large telomeric addition of a block of heterochromatin occurs on pair 12. This is the first report of a chromosomal polymorphism of this nature in amphibians and is probably analagous to the supernumerary block systems observed in many orthopteran insects (Shaw, 1971; John and King, 1977; King and John, 1979).

In the same species, fixed differences due to the addition of procentric blocks on chromosome pairs 3 and 4 occur (see Figs. 12 and 13). In these instances the relative chromosome arm lengths are modified to the same degree as the amount of C-banded chromatin added. Other instances of chromosome addition involving procentric blocks include *L. chloris* (pair 1 and pair 9, short arms), *L. phyllochroa* and *L. pearsoni* (chromosome pair 12 both arms and *L. olongburensis* (pair 5 long arm).

b) Transformation of Euchromatin

In a number of *Litoria* species whole arm C-band blocks are present in the karyotype yet their inclusion has not produced a corresponding modification in relative chromosome size. In those cases were such a C-block is associated with a secondary constriction (such as pair 10 in *L. lesueuri*) chromosome arm lengths are changed but this appears to be due to the presence of the constriction itself. The amount of non C-banding material in these arms is thus reduced. In fact, many of the karyotypic differences between species of *Litoria* involve such major C-blocks and these are clearly grounds for arguing that the process involved in their production is one of euchromatin transformation. This argument can be supported on three grounds:

(1) The genus *Litoria* is a complex of closely related species which share the same basic karyotype in terms of external chromosome morphology. This feature is emphasized by the retention of certain C-bands common to all species, for example the presence of a large grey telomeric block on the long arm of chromosome pair 3 (Fig. 12). When major block differences of the transformation type are present they appear as derived forms. That is, most species have predominantly euchromatic arms for chromosome pair 4 and only a few have either the long *(L. infrafrenata)* or the short *(L. peronii)* arm of this particular chromosome completely C-banded. This suggests that the transformation process is an evolutionary derived state, i.e. it involves a change from euchromatin

into heterochromatin. The fact that completely different chromosomes in different species exhibit such a contrast in form supports the concept of transformation. Thus pair 6 in *L. adelaidensis*, pairs 4, 6 and 8 in *L. peroni*, pair 7 in *L. raniformis* and pairs 4, 7 and 8 in *L. infrafrenata* fall into this category.

(2) Perhaps the best evidence for euchromatin transformation comes from a comparison of sister species. In a number of cases very closely related species have major block differences of this type. In the *L. aurea* complex (Tyler and Davies, 1978), of which *L. raniformis* and *L. moorei* are members, *L. raniformis* is distinguished by possessing a large C-banded block on the long arm of chromosome pair 7 (Figs. 2 and 12). Both *L. aurea* (King unpublished) and *L. moorei* lack this block, yet chromosome pair 7 is the same relative size in the three species. Comparable cases are seen in the *L. peroni* species group (Tyler and Davies, 1978) in which *L. peroni* possesses blocks in 4, 6 and 8, which are absent in the sister species *L. rothi* (King unpublished). Similarly in the *L. bicolor* complex (Tyler and Davies, 1978), of which *L. cooloolensis* and *L. olongburensis* are members, it is only the latter which possesses blocks on pairs 6, 8, 9 and 11 (Fig. 12). In all these cases relative chromosome dimensions are unaltered in the karyotype.

(3) Apart from the above mentioned major blocks, most of which appear to have a procentric origin, there are numerous minor procentric blocks which vary in size between species and are also probable transformation products.

If the C-banded blocks observed are not the product of euchromatin transformation, then the only alternative would be to suppose that the ancestral species of *Litoria* had a range of karyotypes differing in arm numbers, all of which acrued additional segments to produce the very uniform karyotypes that we see today. In a number of cases this would necessitate that the ancestral karyotypes included telocentric chromosomes which were subsequently converted to metacentricity by the addition of heterochromatic arms. This hypothesis implies a polyphyletic origin for the most closely related species of *Litoria* which is an unrealistic proposition because there are clear species pairs in this genus. There are two further complications to a model based on addition. First, when genomic addition has obviously occurred the products are readily recognizable (e.g., *L. meiriana*). Second, a number of other genera (and in some cases, families) of anurans are known to have an all biarmed karyotype with $2n=26$ but without any major C-banded blocks being present (Schmid, 1978a, b).

There are 5 additional cases which support the concept of euchromatin transformation. First, in his study on hylid, bufonid, microhylid, rhacophorid and ranid species, Schmid (1978a, b) found numerous species which were karyotypically uniform but had unaccountable, and usually small, interstitial C-bands which did not alter the proportions of the members of the karyotype. Schmid himself suggested that they were in fact deactivated centromeres of smaller chromosomes, which had been translocated onto larger elements. The situation could just as easily have been interpreted as a series of euchromatin transformations, without the need to invoke such a dubious mechanism as multiple whole chromosome translocations.

Second, in her analysis of Austrian populations of the closely related *Rana ridibunda*, *Rana lessonae* and *Rana esculenta*, Heppich (1978) confirmed previous electrophoretic and morphological studies showing that *R. esculenta*

is of a hybrid origin. She also shows a large C-block in the short arm of pair 11 in *R. lessonae* which is not found in *R. ribibunda*. The hybrid *R. esculenta* is said to be regularly heterozygous although Schmid (1978) failed to confirm the presence of this block. The fact that both homologues in the heterozygote have an identical size indicates that if the block is indeed present then its origin too must depend on euchromatin transformation.

Third, Singh et al. (1976) proposed that the homomorphic but distinctly C-banded W chromosome found in the colubrid snake *Ptyas mucosus* is derived from a homomorphic and non C-banded chromosome in the boids. They also argue that this chromosome became heterochromatinized by a series of pericentric inversions which spread C-banding material associated with a particular DNA satellite throughout the W. However, there is no evidence from external chromosome morphology suggestive of any such series of pericentric inversions; the centromere position remains unchanged in this chromosome. Indeed, it would appear most reasonable to suggest that this is also a case of euchromatin transformation.

A fourth case supporting the concept of euchromatin transformation is the presence of a heteromorphic and sex linked C-banded block on chromosome 6 in a single population of the frog *Rana clamitans* (Mengden, pers. comm.). In this species chromosome arm lengths remain the same but female specimens possess a major block on one homologue on the long arm of pair 6 which is absent in the male. That is, in one homologue of the female pair 6, the euchromatin appears to have been transformed to heterochromatin.

Fifth, White (1973) refers to numerous cases of neo XY systems in Orthoptera where transformation of euchromatin into heterochromatin has been plausibly claimed. For example, in the undescribed morabine grasshopper P45b the entire length of the neo X is heterochromatic despite the fact that its proximal region is autosomal in origin. Clearly, transormation of the euchromatic autosomal material has occurred since the origin of this sex chromosome system.

A mechanism which permits the transformation of euchromatic areas to heterochromatin necessarily provides a means of stabilizing or "locking up" major gene complexes by preventing recombination in their vicinity. This would be particularly useful in those cases where secondary constrictions are newly amplified with heterochromatinization occurring around these sites. Similarly, heterologous sex regions can also be effectively isolated by such a transformation process (King, 1977).

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References

Barker, J., Grigg, G.: A field guide to Australian frogs. Rigby: Australia Ltd (1977)

Bianchi, N.O., Bianchi, M.S., Vidal-Rioja, L.: Heterochromatin later replication and secondary constrictions in the chromosome complement of Leptodactylus ocellatus. Caryologia (Firenze) **26**, 397–403 (1973)

Bicudo, H.E.M.C., Richardson, R.H.: Gene regulation in Drosophila mulleri, Drosophila arizonensis and their hybrids: the nucleolar organizer. Proc. nat. Acad. Sci. (Wash.) **74**, 3498–3502 (1977)

Bogart, J.P.: Karyotypes. In: Evolution in the genus Bufo. Austin: W.F. Blair (ed.). University of Texas Press 1972

Bogart, J.P.: Evolution of anuran karyotypes. In: Evolutionary biology of the anurans. (J.L. Vial, ed.) University of Missouri Press 1973

De Weese, J.: Chromosomes in Eleutherodaytylus (Anura, Leptodactylidae). Mammal. Chrom. Newsletter **16**, 121–123 (1975)

Goodpasture, C., Bloom, S.E.: Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma (Berl.) **53**, 37–50 (1975)

Gropp, A., Winking, H., Zech, L., Müller, H.: Robertsonian Chromosomal variation and identification of metacentric chromosomes in feral mice. Chromosoma (Berl.) **39**, 265–288 (1972)

Heppich, S.: Hybridogenesis in Rana esculenta: C-band karyotypes of Rana ribibunda, Rana lessonae and Rana esculenta. Z. Zool. Syst. Evolutionsforsch. **16**, 27–39 (1978)

Hutchinson, N., Pardue, M.L.: The mitotic chromosomes of Notophthalmus (=triturus) viridescens: localization of C banding regions and DNA sequences complementary to 18 S, 28 S and 5 S ribosomal RNA. Chromosoma (Berl.) **53**, 51–69 (1975)

John, B., King, M.: Heterochromatin variation in Cryptobothrus chrysophorus. 1. Chromosome differentiation in natural populations. Chromosoma (Berl.) **64**, 219–239 (1977)

King, M.: The evolution of sex chromosomes in lizards. In: Evolution and reproduction. Proc. 4th Int. Conf. on Reproduction and Evolution, pp. 55–60. Aust. Acad. Sci. 1977

King, M., John, B.: Regularities and restrictions governing C-band variation in Acridoid grasshoppers. Chromosoma (Berl.) **76**, 123–150 (1980)

King, M., King, D.: Chromosomal evolution in the lizard genus varanus (Reptilia). Aust. J. Biol. Sci. **28**, 89–108 (1975)

King, M., Rofe, R.: Karyotypic variation in the Australian gekko Phyllodactylus marmoratus (Gray) (Gekkonidae: Reptilia). Chromosoma (Berl.) **54**, 75–87 (1976)

King, M., Tyler, M.J., Davies, M., King, D.: Karyotypic studies on Cyclorana and associated genera of Australian frogs. Aust. J. Zool. **27**, 373–393 (1979)

Macgregor, H.C., Kezer, J.: The nucleolar organizer of Plethodon C. cinereus (Green). 1. Location of the nucleolar organizer by in situ nucleic acid hybridization. Chromosoma (Berl.) **42**, 415–426 (1973)

Macgregor, H.C., Mizuno, S.: In situ hybridization of "Nick translated" ^{3}H-ribosomal DNA to chromosomes from salamanders. Chromosoma (Berl.) **54**, 15–25 (1976)

Macgregor, H.C., Vlad, M., Barnett, L.: An investigation of some problems concerning nucleolus organizers in Salamanders. Chromosoma (Berl.) **59**, 283–299 (1977)

Menzies, J.I., Tippet, J.: Chromosome numbers of Papuan Hylid frogs and the Karyotype of Litoria infrafrenata (Amphibia Anura, Hylidae). J. Herpetol. **10**, 167–173 (1976)

Morescalchi, A.: Amphibia. In: Cytotaxonomy and vertebrate evolution. (A.B. Chiarelli and E. Capanna, eds.). pp. 233–348. New York and London: Academic Press 1973

Nardi, I., Barsacci-Pilone, G., Batistoni, R., Andronico, F.: Chromosome location of the ribosomal RNA genes in Triturus vulgaris meridionalis (Amphibia, Urodela). 11. Intraspecific variability in number and position of the chromosome loci for 18 S and 28 S ribosomal RNA. Chromosoma (Berl.) **64**, 67–84 (1977)

Rothfels, K., Freeman, M.: The salivary gland chromosomes of three North American species of Tarinnia (Diptera: Simuliidae). Canad. J. Zool. **44**, 937–945 (1966)

Rudak, E., Callan, H.G.: Differential staining and chromatin packing of the mitotic chromosomes of the newt Triturus cristatus. Chromosoma (Berl.) **56**, 349–362 (1976)

Schmid, M.: Chromosome banding in Amphibia. 1. Constitutive heterochromatin and nucleolus organizer regions in Bufo and Hyla. Chromosoma (Berl.) **66**, 361–388 (1978a)

Schmid, M.: Chromosome banding in Amphibia. 11. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae, Rhacophoridae. Chromosoma (Berl.) **68**, 131–148 (1978b)

Shaw, D.D.: The supernumerary segment system of Stethophyma. 1. Structural basis. Chromosoma (Berl.) **30**, 326–343 (1970)

Singh, L., Purdom, I.F., Jones, K.W.: Satellite DNA and the evolution of sex chromosomes. Chromosoma (Berl.) **59**, 43–62 (1976)
Stephenson, E.M., Stephenson, N.G.: Karyotypes of two Australian hylids. Chromosoma (Berl.) **30**, 38–50 (1970)
Tyler, M.J., Davies, M.: Species groups within the Australopapuan hylid frog genus Litoria tschudi. Aust. J. Zool., Suppl. series, **63** (1978)
Varley, J.M., Morgan, G.T.: Silver staining of the lampbrush chromosomes of Triturus cristatus carnifex. Chromosoma (Berl.) **67**, 233–244 (1978)
Ward, O.G.: Dimorphic nucleolar organizer regions in the frog Rana blairi. Canad. J. Cytol. **19**, 51–57 (1977)
White, M.J.D.: Animal cytology and evolution, 3rd edit. London: Cambridge University Press 1973
White, M.J.D.: Chromosome repatterning: regularities and restrictions. Genetics **79**, 63–72 (1975)
Wilson, A.C., Sarich, V.M., Maxson, L.R.: The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. Proc. nat. Acad. Sci. (Wash.) **71**, 3028–3030 (1974)

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