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Karyotype, C-Band and Ag-Nors Study of Three Stink Frogs

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Abstract.-The karyotypes,C-bands and Ag-NORs of *Rana kuangwuensis, R.andersonii* and *R.margaratae* were analyzed. Intra- and interspecific chromosome variations, including centromeric type and C-banding patterns, were detected. It was assumed that the Guizhou Plateau was the distributional center of the original place of the group.

Key Words: Amphibia, Ranidae, *Rana kuangwuensis, Rana andersonii, Rana margaratae*, China, karyotype, C-band, Ag-NORs.

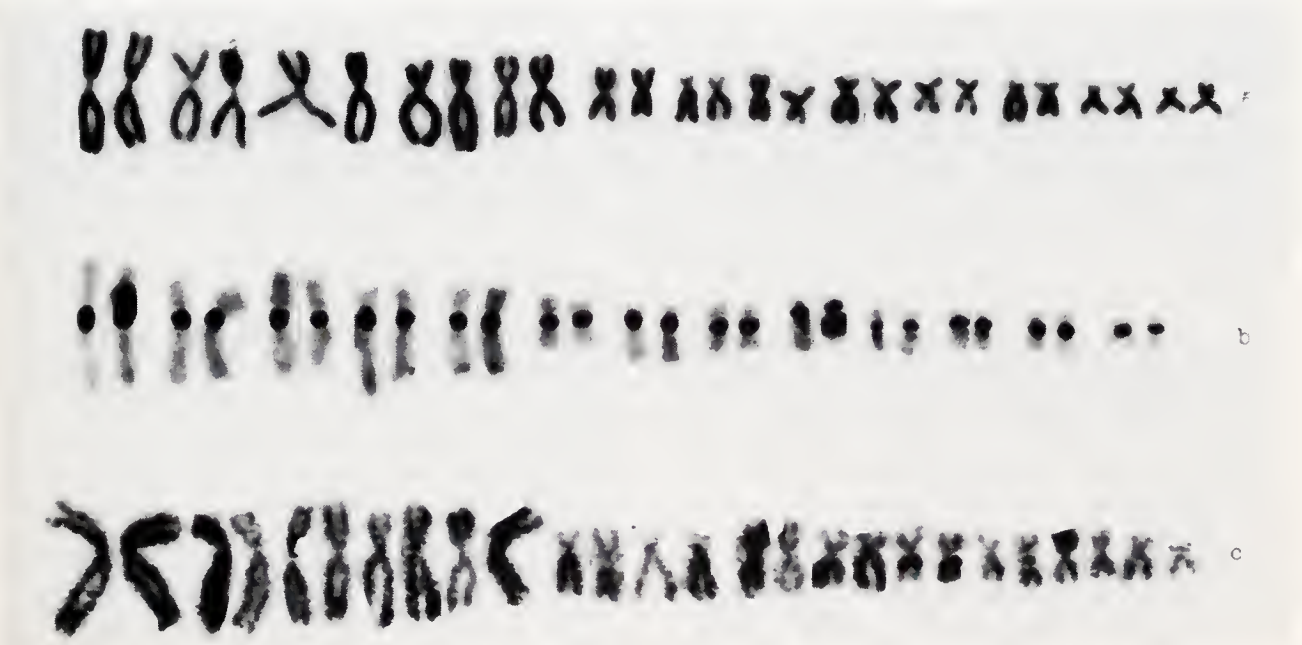

FIG. 1. a: Karyotype of *Rana andersonii.* b: showing C-bands. c: showing Ag-NORs.

Introduction

The group of stink frogs which have a special stink smell from the skin consists of nine species, i.e. Rana andersonii, R. anlungensis, R. grahami, R. kuangwuensis, R. lungshengensis, R. margaratae, R. schmackeri, R. tiannanensis and R. wuchuanensis. They are considered to be phylogenetically close because of similar morphological characters in adults and tadpoles. Among them, the karyotype and C-bands of R. grahami from Kunming, Yunnan has been studied by Li (1982). In addition, the karyotype, C-bands and Ag-NORs of R. margaratae from Emei Mountain, Sichuan have been analyzed by Wang (1983) and Wu (1990). In the present paper, the karyotypes, C-bands and Ag-NORs of R. kuangwuensis, R. andersonii and R. margaratae were analyzed.

Methods

Two females and one male *R. andersonii* were captured at Qianxi, Guizhou Province, (27°20' N,106°16' E). One female and three male *R. kuangwuensis* were captured at Nanjiang, Sichuan Province (32°30' N, 106°40' E) and one female and

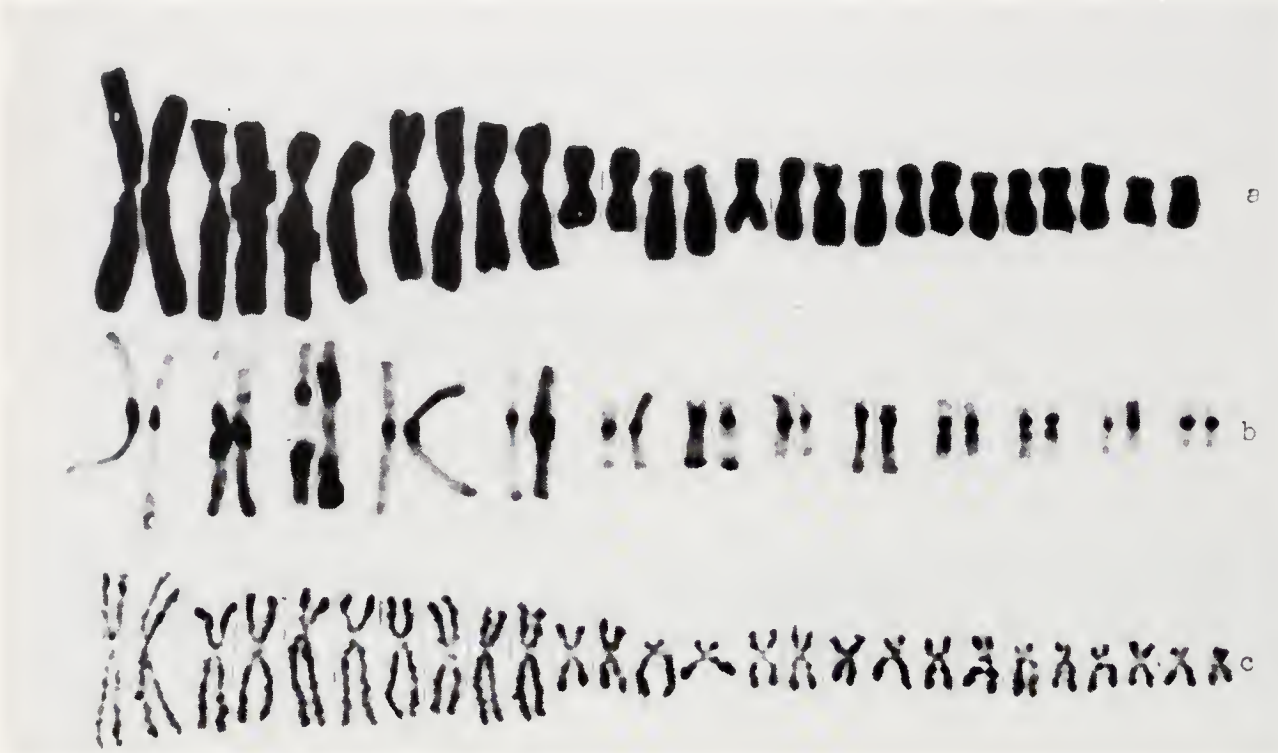

FIG. 2. a: Karyotype of *Rana kuangwuensis*. b: showing C-bands. c: showing Ag-NORs.

one male *R. margaratae* from Zunyi (27°40' N, 106°50' E) were captured. Karyotypes, C-bands and Ag-NORs preparations were made after Wei et al. (1990).

Results

Figures 1, 2, and 3 depict the karyotypes, C-bands and Ag-NORs of *R. andersonii*, *R. kuangwuensis* and *R. margaratae*. For the measurment of the karyotypes see table 1. The diploid number of the species are all the same, 2n=26, comprising two groups.

The large chromosome group includes chromosome Nos. 1-5, with a relative length (R.L.) larger than 9%. With regard to the arm ratio (A.R.), chromosome Nos. 1 and 5 are metacentric in all three species. No. 2 is metacentric in *R. margaratae* and *R. andersonii*, but submetacentric in *R. kuangwuensis*. No.3 is submetacentric in *R. margaratae* and *R. kuangwuensis* , but metacentric in *R. andersonii*. No.4 is submetacentric in *R. andersonii* and *R. margaratae*, but metacentric in *R. kuangwuensis*.

The small chromosome group comprises chromosome Nos. 6-13, with a R.L. less than 7%. Nos.6, 8, 10, 12 and 13 are metacentric, No.7 is submetacentric in all the three species. Nos. 9 and 11 are submetacentric in *R. kuangwuensis* and *R. andersonii* but metacentric in *R. margaratae*. Secondary constrictions are observed in the long arms of No. 10 of *R. margaratae* (only one homologous) and *R. andersonii* but not observed in *R. kuangwuensis*.

Treatment of the chromosome of the three species according to the C-banding method shows that each species has a centromeric C-band on each chromosome. For interstitial C-band, it is quite different among the species. There is only an interstitial C-band in 10q in *R. andersonii*. And there is an interstitial C-band in 2p (stained weakly), 3q and 4q (only one homologous) in *R. margaratae*. But there are much more interstitial C-bands in *R. kuangwuensis* than in the other two species. *R. margaratae* and *R. andersonii* have not any telomeric C-band. But *R. kuangwuensis* has some telomeric C-bands.

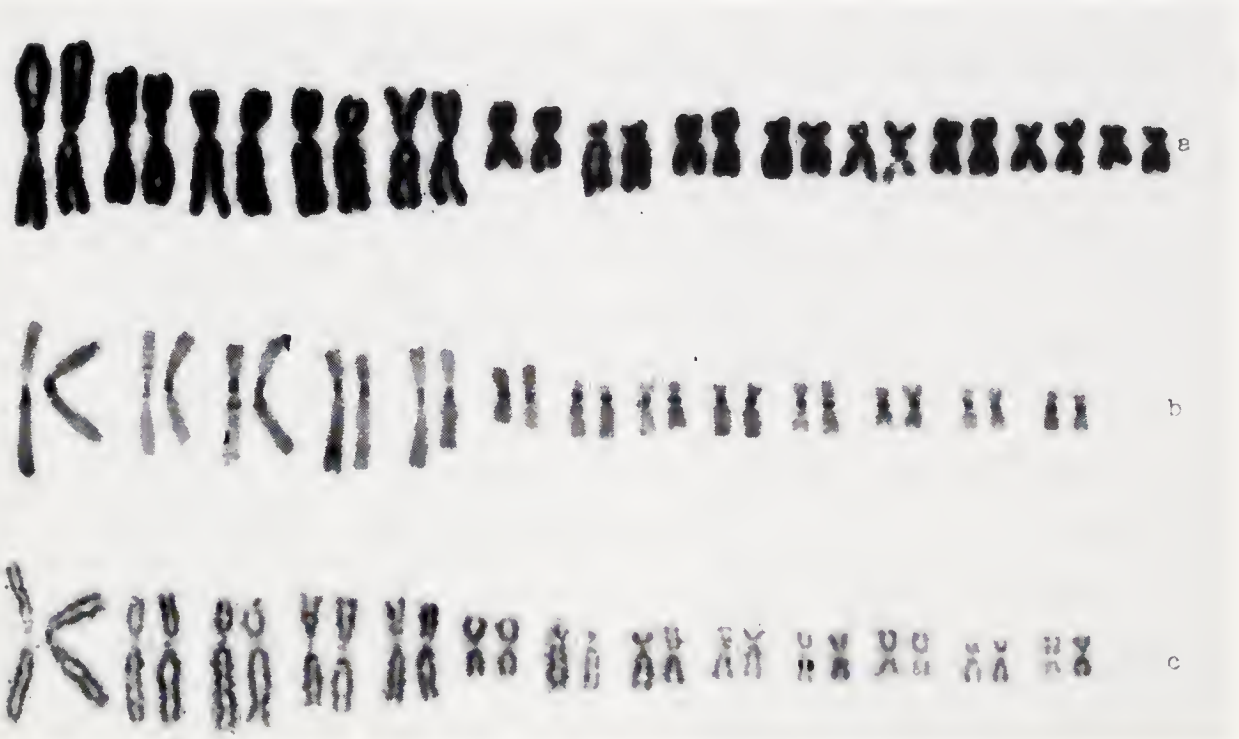

FIG. 3. a: Karyotype of *Rana margaratae*. b: showing C-bands. c: showing Ag-NORs.

Specific staining of the NORs with silver (Ag) confirms that the regions of NORs in all the three species are the same, in the long arms of chromosome No.10. But the relation between the NORs and the constrictions is quite different. The regions of the one pair of NORs in *R. andersonii* correspond to the regions of the secondary constritions. *R. margaratae* has only one NORs in the chromosome where the secondary constriction locates, and no NORs is observed in the other homologous which has no secondary constriction. While for *R. kuangwuensis*, no secondary constriction is observed in the regions of the NORs.

Discussion

Comparing the karyotypes between the three species, we detect some interspecific variations. The secondary constriction is not detected in *R. kuangwuensis*. The chromosomes consist of 8 metacentric and 5 submetacentric pairs in *R. kuangwuensis* and *R. margaratae*, while 9 metacentric and 4 submetacentric in *R. andersonii*.

C-banding patterns of all 13 pairs of *R. margaratae* are similar to those of *R. andersonii*, except for the variant bands on chromosomes 2, 3, 4, and 10. While C-banding pattern of *R. kuangwuensis* is quite different from those of the other two species, for *R. kuangwuensis* has more interstitial C-bands and telomeric C-bands as well. And the relation between the NORs and the secondary constrictions is quite different among the three species.

This study also indicates that intraspecific chromosome variations exist in *R. margaratae* from different distributional areas. We observe 8 metacentric and 5 submetacentric pairs in the present study, as opposed to 12 metacentric and 1 submetacentric pairs from Emei Mountain, Sichuan (Wang et al., 1983) and 9 metacentric and 4 submetacentric pairs also from Emei Mountain (Wu 1990).

The C-banding pattern of chromosomes 1-13 of *R. margaratae* from Zunyi is compared with those from Emei Mountain. There is a centromeric C-band in each chromosome and a terminal C-band at each chromosome terminus, and an interstitial C-band in the long arm of No.3 from Emei

TABLE 1. Karyotypic data for *Rana margaratae*, *R. andersonii*, and *R. kuangwuensis*.

Chromosome Nos.	*R. margaratae*	*R. andersonii*	*R. kuanguensis*
1	R. L. 14.64±0.83	R. L. 14.30±0.91	R. L. 15.70±0.91
	A. R. 1.26±0.31	A. R. 1.26±0.33	A. R. 1.46±0.26
2	R. L. 12.38±0.84	R. L. 11.90±0.70	R. L. 13.11±0.32
	A. R. 1.48±0.38	A. R.1.43±0.35	A. R. 1.54±0.41
3	R. L. 11.73±0.86	R. L. 11.20±0.68	R. L. 11.93±0.94
	A. R. 1.72±0.49	A. R. 1.57±0.28	A. R. 2.01±0.39
4	R. L. 11.39±0.77	R. L. 10.21±0.49	R. L. 11.42±0.92
	A. R. 1.76±0.39	A. R. 1.71±0.41	A. R. 1.29±0.39
5	R. L. 10.20±0.84	R. L. 9.58±0.34	R. L. 10.25±0.84
	A. R. 1.39±0.45	A. R. 1.33±0.20	A. R. 1.41±0.37
6	R. L. 6.52±0.45	R. L. 6.82±0.48	R. L. 6.59±0.65
	A. R. 1.21±0.35	A. R. 1.32±0.29	A. R. 1.20±0.16
7	R. L. 5.65±0.37	R. L. 6.09±0.61	R. L. 5.92±0.41
	A. R. 2.53±0.37	A. R. 2.14±0.30	A. R. 2.54±0.39
8	R. L. 5.44±0.48	R. L. 6.01±0.60	R. L. 5.40±0.35
	A. R. 1.39±0.38	A. R. 1.38±0.46	A. R. 1.24±0.37
9	R. L. 5.27±0.42	R. L. 5.73±0.57	R. L. 5.13±0.27
	A. R. 1.92±0.34	A. R. 2.06±0.39	A. R. 2.01±0.36
10	R. L. 4.73±0.43	R. L. 5.30±0.53	R. L. 4.87±0.32
	A. R. 1.35±0.26	A. R. 1.27±0.25	A. R. 1.55±0.43
11	R. L. 4.39±0.38	R. L. 4.92±0.32	R. L. 4.34±0.36
	A. R. 1.32±0.36	A. R. 1.57±0.42	A. R. 1.92±0.37
12	R. L. 4.28±0.56	R. L. 4.56±0.41	R. L. 4.21±0.32
	A. R. 1.27±0.29	A. R. 1.53±0.37	A. R. 1.45±0.37
13	R. L. 3.98±0.34	R. L. 4.12±0.39	R. L. 3.55±0.40
	A. R. 1.47±0.39	A. R. 1.66±0.33	A. R. 1.66±0.35

Mountain (Wang et al , 1983). Besides those above, there is an interstitial C-band in the acro long arm of No.7, and even heterogeneity observed in No.9. There is an interstitial C-band in the middle of the long arms of both homologues of No.9 in female, while only one homologous of No.9 is observed having an interstitial C-band, the other homologue has not an interstitial C-band in the middle of the long arm, but has an interstitial C-band near the terminus of the long arm (Wu 1990). We also observed indeed C-band heterogeneity of chromosome No.9 from Emei Mountain. Yet in our present study, we do not detect C-band heterogeneity of chromosome No.9 from Zunyi. And we detect other interstitial C-band (2p, 4q) but no telomeric C-band has been observed.

In the early stage of the karyotypic evolution, a karyotype had generally more metacentric chromosomes. With the development, the karyotype differenciated in the direction of having more submetacentric or telocentric chromosomes (Li, 1985). Generally speaking, karyotypes with more telomeric and less interstitial C-band are more original. Between the two distributional areas of *R.margaratae*, the specimens from Emei Mountain has 12 (Wang 1983) or 10 (Wu, 1990) metacentric, and has more telomeric and less interstitial C-bands, and that from Zunyi has 8 metacentric and has less telomeric and more interstitial C-bands. On the view of point above, *R.margaratae* from Emei Mountain is more original than that from Guizhou.

The stink frog group is composed of 9 species. The distributions of them are as follows:

R. andersonii: upper Burma to Yunnan, Guizhou, Guangxi, Hainan

R. anlungensis: Guizhou (Anlung County)

R. grahami: Sichuan, Guizhou, Yunnan

R. kuangwuensis: Sichuan (Nanjiang County)

R. lungshengensis: Guizhou, Guangxi, Hunan

R. margaratae: Gansu, Sichuan, Guizhou

R. schmackeri: Henan, Gansu, Sichuan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Jiangxi,Hubei, Guangdong

R. tiannanensis: Yunnan, Hainan

R. wuchuanensis: Guizhou (Wuchuan, Libo)

From the discription above, it could be found that the distributional areas of some species are very limited, only one or two counties. So they are very rare and precious wildlife. And it could also be found that the stink frog group is distributed mainly in the south of China, and most of them (7 species) are found on the Guizhou Plateau. So, the Guizhou Plateau might be the distributional center of the group.

There were another two species of stink frogs. Their karyotypes and C-banding patterns were published. They are *R. grahami* and *R. schmackeri*. Both the species have 10 metacentric and 3 submetacentric pairs in their karyotypes, and both species have one telomeric C-band, but the former has 5 and the latter has 4 interstitial C-bands.

Among the 5 species published their karyotypes and C-banding patterns, *R. margaratae* from Emei Mountain has most metacentric pairs and most telomeric C-bands. Although it does not have less interstitial C-bands, it could still be considered as the most original in the viewpoint of cytogenetics. Considering that *R. margaratae* from Guizhou is more evoluted than that from Emei Mountain, it might be assumed that the stink frog group originated in Emei Mountain and its adjacent plateau.

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