Localization of NORs in karyotypes of four *Rana* species

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Abstract

NORs were localized by Ag-AS-staining in karyotypes of *Rana nigromaculata, R. macrocnemis, R. latastei* (2n = 26) and *R. chensinensis*, 2n = 24. As in the majority of other *Rana* species, the NORs are located in the long arms of the 10th (or 11th in *R. chensinensis*) chromosome pair. The presence of a second secondary constriction in *R. latastei,* as reported earlier, was not confirmed.

Introduction

The presence and position of secondary constrictions which are potentially the nucleolus organizer regions (NORs, the sites of 18S + 28S rDNA) are important characteristics in karyosystematics. With regards to amphibian karyotypes, especially those of *Rana* species, secondary constrictions were discussed by Hennen (1964), Seto (1965), Guillemin (1967), Ullerich (1967), Günther (1970), Nishioka (1972), Kuramoto (1972, 1980), Kuramoto *et al.*, (1973), Ivanov and Madyanov (1973), Belcheva and Genova (1974), Haertel *et al.* (1974), Popov (1974), Orlova *et al.* (1977), Koref-Santibanez and Günther (1980), Green *et al.* (1981), Green (1983). NORs can be specifically stained by the Ag-AS-method (Goodpasture & Bloom, 1975), although some other chromosome sites are stained during this procedure too (Nardi *et al.*, 1978; Vitelli *et al.*, 1982; Bailly, 1983).

As a rule, intensively Ag-stained regions of chromosomes correspond to secondary constrictions; in some cases, however, data published by authors who used the Ag-staining method and those who did not, were different. For instance, the results of Ag-staining of *Bufo* chromosomes (Schmid, 1978a; Beck & Mahan, 1979; Birstein, 1981a) point out that Bogart (1972) perhaps overestimated the number of secondary constrictions; in fact, NORs (and the corresponding secondary constrictions) are located in homologues of only one *Bufo* chromosome pair – the 1st, 6th or 11th. Previously chromosomes of some *Rana* species were Ag-stained (Ward, 1977; Schmid, 1978b; Belcheva *et al.*, 1981; Birstein, 1981a, b). This paper describes the position of NORs in karyotypes of four more *Rana* species and discusses the localization of NORs in *Rana* karyotypes in general.

Material and methods

One species of green frog, *Rana nigromaculata* (2♀, 2♂ from Karamet-Niyaz, Turkmenian SSR), and three brown frog species, *R. macrocnemis* (1♀, 1♂ from the Caucasian Wild Life Reserve, Guseripl), *R. latastei* (2♀ from the environments of Mantova, Bosco della Fontana, Italy – Orlova *et al.*, 1977) and *R. chensinensis* (3♂ from the environments of Yujnosakhalinsk, Sakhalin island) were investigated. Chromosome preparations were made from bone marrow cells of colchicinized animals (Aleksandrovskaya *et al.*, 1979). The one-stage method of Ag-AS-staining was used (Howell

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& Black, 1980). One drop of gelatin solution (2% solution in water acidified with formic acid) and two drops of 50% $AgNO_3$ solution were placed on the preparation, then the drops were mixed, and the mixture was covered with a cover glass. The preparation was heated to 70 °C for 2.0–2.5 min, the cover glass taken off and the preparation washed with water, dried and analyzed. In some cases after the preparations had been photographed, silver was removed according to Warburton and Henderson (1979). The slides were first treated with 7.5% sodium ferricyanide (4 min) and after that with a 20% solution of sodium thiosulfate. They were then washed with water and stained with Giemsa.

Results and discussion

Figure 1 shows karyotypes of the four investigated *Rana* species after Ag-staining of the chromosomes. The karyotypes of *R. nigromaculata, R. macrocnemis* and *R. latastei* consist of 26 chromosomes while the *R. chensinensis* karyotype consists of 24 chromosomes; these data coincide with findings of Nishioka (1972), Ivanov and Madyanov (1973) and Orlova *et al.* (1977). On the basis of relative length the chromosomes of the first three species (2n = 26) can be divided into two groups: large chromosomes (pairs 1–5) and small ones (pairs 6–13). All large chromosomes are meta- or submetacentric, while some of the small ones are subtelocentric (e.g. pairs Nos. 7, 9 in the *R. latastei* karyotype). The three karyotypes are very similar and the main difference between *R. nigromaculata* and *R. macrocnemis* and *R. latastei* is the centromeric position in the 4th pair: in *R. nigromaculata* these are almost subtelocentric, in *R. macrocnemis* and *R. latastei* they are submetacentric. It should be noted that we analyzed the karyotypes of *R. nigromaculata* from Central Asia, where this species had been introduced from China (Aleksandrovskaya & Orlova, 1981). The karyotypes of this form and of the Far Eastern *R. nigromaculata* are practically indistinguishable (Aleksandrovskaya & Orlova, 1981).

In all three frog species (2n = 26) NORs are located in the long arms of the homologues of the 10th chromosome pair (Fig. 1). In *R. nigromaculata* and *R. latastei* these are submetacentric and NORs are situated about the middle of the long arms; in *R. macrocnemis* they are almost metacentric and NORs are located in the long arms near the centromeres.

Orlova *et al.* (1977) described two secondary constrictions (one in each arm) in the 7th pair of *R. latastei.* According to our numeration, this pair could be No. 10. However, we did not find any additional constriction in the small chromosomes, neither after Ag-staining nor after silver had been removed and the same preparations were stained with Giemsa. Thus, the 2nd secondary constriction described by Orlova *et al.* (1977), could be the result of an artefact.

There is little difference in the length of *Rana* chromosomes Nos 7–11 (and especially Nos. 9–11). That is why several authors gave different numbers to the chromosomes having secondary constrictions (e.g. Kuramoto, 1972, and Kuramoto *et al.*, 1973, reported secondary constrictions in the 9th pair of the Far Eastern *Rana* species, instead of in the 10th). Some authors did not find constrictions at all. For instance, secondary constrictions previously were not found in *R. macrocnemis* (Ivanov & Madyanov, 1973), while we localized the NORs in the 10th pair of this species.

The difference in length of large and small chromosomes of *R. chensinensis,* 2n = 24, is not as evident as in *Rana* species with 2n = 26. The chromosomes grade smoothly as in the case of *R. dybowskii* and *R. ornativentris* (Green, 1983). NORs of *R. chensinensis* are located in the long arms of the homologues of one of the smallest pairs, which we designate by No. 10, as in another Ag-stained *Rana* species, 2n = 24, *R. macrodon* (Schmid, 1980a). In the related species, *R. dybowskii,* this pair was designated as No. 11 (Green, 1983).

The karyotype of *R. chensinensis* is very similar to that of *R. dybowskii* (Green, 1983), there are two pairs of submetacentrics, Nos. 8 and 10 (11 in Fig. 1) in these two karyotypes, which probably originated after an initial chromosome number reduction from a 26-chromosome ancestor (Green, 1983). The C-band pattern of *R. dybowskii* suggested to Green (1983) that the 24-chromosome karyotype could arise by a fusion of two small chromosome pairs in the karyotype of a 26-chromosome ancestor. A new chromosome of intermediate size (No. 6) derived from this fusion would shift the small NOR-bearing chromosome from its

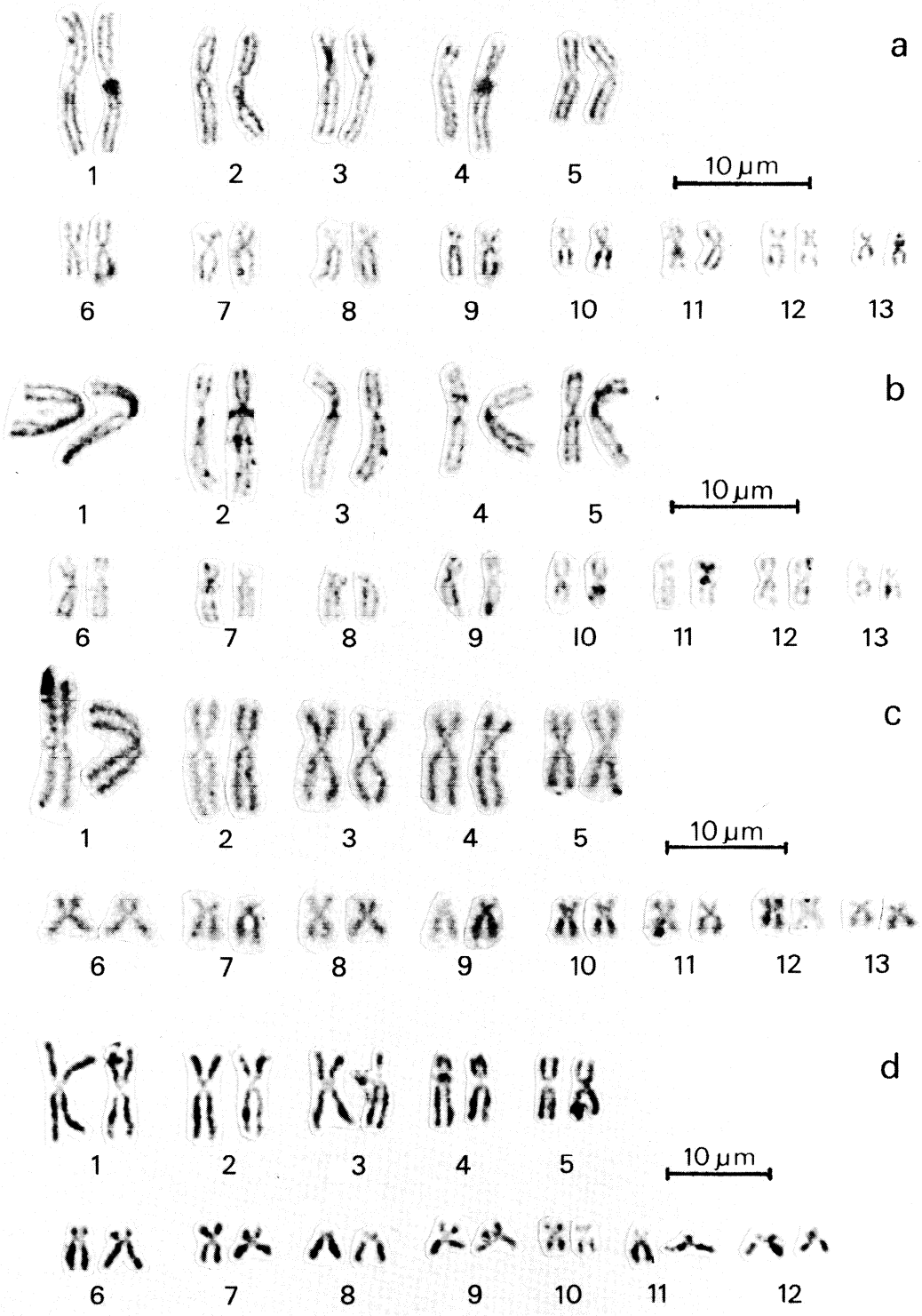

Fig. 1. Karyotypes of *Rana nigromaculata* (a), *R. macrocnemis* (b), *R. latastei* (c), and *R. chensinensis* (d). Chromosomes were stained by the Ag-AS method; the homologues of the 10th pairs have Ag-positive NORs in the long arms.

Table 1. The localization of nucleolus organizer regions in karyotypes of Ranidae (Ag-positive sites).

Species	Origin	2n	Position of Ag-NOR[a]	References
Rana esculenta	Central Europe	26	10q[b]	Schmid, 1978b; Birstein, 1981a
R. graeca	Western Europe	26	10q[c]	Vitelli *et al.*, 1982
R. latastei	Western Europe	26	10q	This paper
R. lessonae	Central Europe	26	10q	Birstein, 1981a
R. ridibunda	Central Europe	26	10q	Schmid, 1978b; Belcheva *et al.*, 1981; Birstein, 1981a, b
R. temporaria	Central Europe	26	10q[b]	Schmid, 1978b; Birstein, 1981a
R. macrocnemis	The Caucasus	26	10q	This paper
R. erythrea	Southeast Asia	26	10q (6q, 8q, 8p, 11p, 13p	Schmid, 1978b; 1980a
R. nigromaculata	Central Asia	26	10q	This paper
R. chensinensis	Far East	24	10q[d]	This paper
R. macrodon	Southeast Asia	24	10q	Schmid, 1980a
R. blairi	North America	26	10q	Ward, 1977
R. catesbeiana	North America	26	10q[b] (8p, 12p[e])	Schmid, 1978b
R. magnaocularis	North America		10q	Ward, 1977
R. palustris	North America	26	10q (6q, 6p, 7p, 7q, 12p, 13q)	Schmid, 1978b; 1980a
R. sphenocephala	North America	26	10q (9p, 13p)	Schmid, 1978b
Pyxicephalus adspersus	South Africa	26	6p (8p)	Schmid, 1978a, b
P. delalandii	South Africa	26	13p	Schmid, 1980b

[a] q: long arm; p: short arm; the additional Ag-positive sites (not NORs) are listed in brackets.
[b] The localization of NORs was confirmed by *in situ* hybridization of ^{3}H-18S-28S rRNA (Vitelli *et al.*, 1982).
[c] NOR was localized only by *in situ* hybridization experiments (Vitelli *et al.*, 1982).
[d] This pair is No. 11, but it is homologous to the pair No. 10 in the 26-chromosome *Rana* species (Green, 1983).
[e] This site appears to correspond to the 5S locus (Vitelli *et al.*, 1982).

usual tenth position in the 26-chromosome karyotypes to the eleventh position in the 24-chromosome *R. chensinensis* and *R. dybowskii* karyotypes. The same shift has probably occurred in the karyotype of *R. ornativentris* (Green, 1983).

Thus NORs are located in the 10th (or in the homologous 11th) chromosome pairs of four investigated *Rana* species. The same is true for many other *Rana* species (Table 1). Only in African species of *Pyxicephalus,* which are closely related to *Rana,* are NORs located in the 6th or 13th pairs (Schmid, 1980a, b). In the case of *P.adspersus,* there is one additional NOR in the short arm of a small homologue of the heteromorphic pair No. 8 (Schmid, 1980b). It seems possible that this change in the *P. adspersus* karyotype, in comparison with that in *Rana* is connected with considerable chromosome rearrangements. The karyotype of this species differs from the karyotypes of many *Rana* species and from that of *P. delalandii,* 2n = 26, not only in the presence of the heteromorphic pair 8, but also in the presence of two pairs of telocentric chromosomes (10 and 11; Schmid, 1980a, b), which is very unusual for karyotypes of higher anurans (Morescalchi, 1973).

Relocation of NORs possibly also occurred in *R. arvalis* (with 2n = 24 and the secondary constrictions in the short arms of the 2nd pair - Ullerich, 1967) and *R. dalmatina* (with 2n = 26, the secondary constrictions are in the short arms of pair 3 - Guillemin, 1967; Popov, 1974). Chromosomes of these species have not been Ag-stained yet.

Besides the large NORs in the 10th pair, some North American and Asian *Rana* species have a few additional sites in different chromosomes which were positively stained by the Ag-AS-method (Schmid, 1978b; 1980a, b; Table 1). It is not yet clear if rDNA is localized in these additional sites, or if these Ag-positive regions are the sites of 5S DNA and other genes, as in the case of *Triturus vulgaris meridionalis* (Nardi *et al.*, 1978). Moreover, in another urodelan species, *Pleurodeles walt-*

lii, silver preferentially stains the pericentromeric zones of chromosomes, where, after cold treatment of animals, additional secondary constrictions appear; the positions of these zones are matched by the positions of G-, C-, and Q-bands (Bailly, 1983). As for *R. catesbeiana*, it was shown by *in situ* hybridization of ^{3}H-18S + 28S rRNA and ^{3}H-5S rRNA that NORs are located only in the 10th pair, while the 5S DNA locus seems to be situated in Ag-positive sites of the 12th pair (Vitelly *et al.*, 1982).

Unfortunately, the Ag-AS-method has not yet been used in many *Rana* species. Most interesting are the North American *R. virgatipes* (2n = 26), which has secondary constrictions in both arms of the homologues of the 10th pair (Green *et al.*, 1981), and four Far Eastern *Rana* species, *R. subaspera* and *R. holsi*, 2n = 26, with secondary constrictions in two pairs, Nos. 4 and 9 (10) (Kuramoto, 1972), and *R. namiyei* and *R. kuhlii*, 2n = 22, with secondary constrictions in the homologues of the 6th and 14th pairs (Kuramoto, 1972; 1980).

As regards the additional secondary constrictions in the 24-chromosome species, *R. ornativentris* (pairs Nos. 2, 3, 4, and 7 - Seto, 1965), their positions are matched by heterochromatic C-bands without constrictions in *R. dybowskii* (Green, 1983). The position of the only secondary constriction in the short arms of *R. arvalis* chromosomes No. 2 (Ullerich, 1967) is also matched by the position of the C-band in *R. dybowskii* chromosome No. 2 (Green, 1983). As the NOR in *R. dybowskii* is associated with C-positive heterochromatin (Green, 1983), so the NOR in *R. arvalis* has probably been translocated into another C-heterochromatic region on the 2nd chromosome.

Our data and those shown in Table 1 point out that the localization of the NOR in *Rana* karyotypes is very conservative. Apparently less conservativeness was found in *Bufo* species: in *Bufo* karyotypes Ag-positive NORs may be located in at least three chromosome pairs (Schmid, 1978a; Beck & Mahan, 1979; Birstein, 1981a), In amphibians conservativeness of NOR localization may be characteristic of only some of the higher anurans, because even in the karyotypes of closely related species of Urodela (genera *Triturus, Aneides, Plethodon*), NORs are located in different chromosome pairs (Mancino *et al.*, 1977; Macgregor & Sherwood, 1979).

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