chiasma frequency (as can be seen in figure 3) does not necessarily indicate holocentric chromosomes, though the reverse may be true. On the other hand, the effect of ionizing radiations on the chromosomes of *P. brassicae* and other lepidopteran species has almost confirmed that the lepidopteran chromosomes are holokinetic[8]. Whether two types of centromeric organisations, as suggested by Bigger, exist in *P. brassicae,* was not clear. But the presence of monocentric organization, if detected in more lepidopteran forms, may invalidate our earlier ideas about the evolution of the karyotype in Lepidoptera. Suomalainen has already pointed out that in many groups of Lepidoptera the chromosome numbers are less variable than a diffuse centromere would theoretically allow. Thus, one would expect structural rather than merely numerical variation, as in the case of organisms having monocentric chromosomes. More work on recent lines on the lepidopteran forms is therefore well warranted.

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The chromosomes of *Bufo rubropunctatus* and *Bufo chilensis* (Anura, Bufonidae) and other species of the *spinolosus* group[1]

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Summary. The chromosomes of *Bufo rubropunctatus* and *B. chilensis* (on basis of adults) are described for the first time. These chromosomic sets are compared with the karyotypes of *B. spinolosus* and *B. variegatus* and other species of *spinolosus* group. The importance of the secondary constrictions applied to the phylogeny of *Bufo* are discussed.

Bufo rubropunctatus and *Bufo chilensis* belong to the *Bufo spinolosus* group species which is restricted to the South-Western part of South America (from Ecuador to South Chile and Argentina) specially on the Andes[4]. Other species of this group are *B. atacamensis, B. limensis, B. trifolium* and *B. flavolineatus. B. variegatus* has been included in this group, however this possibility is discussed[5,6]. *Bufo rubropunctatus* is an uncommon toad of the humid and cool forests of Southern Chile which is characterized by its ventral pattern (white spots on a black belly). *Bufo chilensis* is a typical inhabitant of the Central Chilean arid steppe (*Acacia caven* association). Bogart[7] analyzed the chromosomes of several species of genus *Bufo.* He described, among others, the karyotypes of *B. spinolosus, B. variegatus* and *B. chilensis.* The chromosomes of this last species were studied on basis of hybrid tadpoles which were produced in combination of *B. chilensis* (from Zapallar, Central Chile) with 5 species of *Bufo* from North America, *B. viridis* from Eurasia and *B. arenarum* and *B. flavolineatus* from South America.

In this paper, the chromosomes of *B. rubropunctatus* are described for the first time, and new karyological information of *B. chilensis* is presented on basis of adult toads from 2 different populations. Also the karyotypes of *B. spinolosus* and *B. variegatus* are redescribed because our chromosomic results, specially on *B. spinolosus,* are not in agreement with Bogart's data[7]. All the karyological information obtained is here employed in the analysis of karyological relationships among the species of the *Bufo spinolosus* complex.

The karyological materials here studied were obtained from: *B. rubropunctatus,* 16 juveniles from Riñinahue (Valdivia province), *B. chilensis,* 5 males and 2 females from Los Angeles (Bio Bio province) and 3 males from Santiago (Santiago province), *B. spinolosus,* 3 males from Malargue (Mendoza, Argentina) and *B. variegatus,* 5 females from Antillanca (Osorno province). All specimens were deposited in the amphibian collection of Instituto de Zoología, Universidad Austral de Chile, Valdivia (IZUA). Methodology and nomenclature are used as described in previous paper[8]. Secondary constructions were named according to Bogart[7]. The karyotypes for each species are shown in the figure and the results of the chromosomes measurements are included in the table.

According to our results, only the karyotype of *B. variegatus* is more in agreement with Bogart's data; however, minute differences were observed. In relation to *B. spinolosus* and *B. chilensis* karyotypes, important karyological differences were found. The karyotype of *B. spinolosus* has been described with 2 secondary constrictions on pair 3[7]; however in 32 mitotic plates of this species we have found only 1 secondary constriction on pair 10. In *B. chilensis* karyotype constrictions E (pair 2), F (pair 3), K (pair 7) and L (pair 11) were seen by Bogart[7]. The specimens of 2 different populations (Santiago and Los Angeles) here examined have only 1 secondary constriction on pair 7 (K). 4 possibilities are advanced to explain the remarkable differences here found.

1. A sibling species could exist into the morphological complex of *B. chilensis,* this cryptic species could be located in Zapallar (Coastal Range, Central Chile) where Bogart's toads were collected.
2. *B. chilensis* from Zapallar population could show a chromosomic difference in relation to the other populations of this species.
3. Another possibility might be that the karyotypes of the tadpoles are different to those of the adults, Beçak[9] demonstrated this fact in the South American tadpoles of *Odontophrynus americanus,* which have 2 more secondary constrictions than the adults; these have only one.
4. The last possibility might be that the hibrid condition of the tadpoles could involve a modification in number of secondary constriction.

For many years, *B. chilensis* was considered as a subspecies of *Bufo spinolosus (B. s. chilensis)*[10,11]. From the serological point of view, Cei[12] proved that *B. chilensis* is a full species,

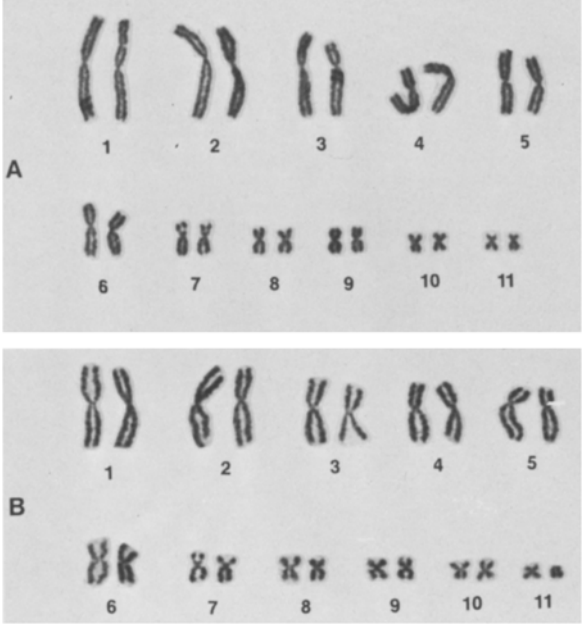
Summary of primary and secondary constrictions and the percentage of the largest chromosome

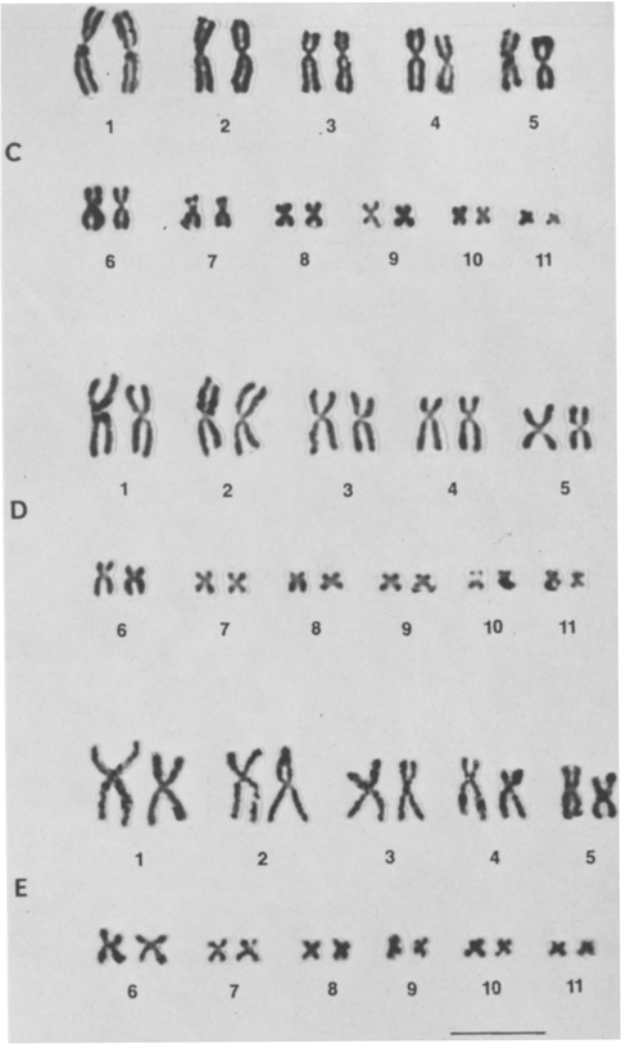
Species		Chromosomes 1	2	3	4	5	6	7	8	9	10	11
Bufo rubropunctatus	r^+	1.2	1.4	1.7	1.5	1.1	1.4	1.4	1.1	1.1	1.0	1.1
	Type	m	m	m	m	m	m	m	m	m	m	m
	%	100	94.6	77.4	64.1	58.6	48.8	33.2	26.7	26.5	20.4	18.3
	C							sm				
B. chilensis	r^+	1.2	1.1	1.1	1.3	1.7	1.1	1.3	1.3	1.1	1.2	1.5
(Santiago)	Type	m	m	m	m	m	m	m	m	m	m	m
	%	100	98.4	74.6	73.4	67.2	52.9	36.0	33.7	29.1	26.3	21.7
	C							sm				
B. chilensis	r^+	1.1	1.2	1.7	1.2	1.2	1.3	1.1	1.0	1.0	1.0	1.2
(Los Angeles)	Type	m	m	m	m	m	m	m	m	m	m	m
	%	100	85.5	70.6	69.5	64.4	52.0	38.1	30.9	30.4	25.5	19.8
	C							sm				
B. spinolosus	r^+	1.2	1.0	1.3	1.7	1.1	1.2	1.0	1.1	1.0	1.0	1.2
	Type	m	m	m	m	m	m	m	m	m	m	m
	%	100	99.8	82.9	75.2	61.9	46.0	34.6	31.8	29.8	29.8	24.7
	C										sm	
B. variegatus	r^+	1.1	1.3	1.2	1.8	1.3	1.2	1.0	1.0	1.1	1.2	1.1
	Type	m	m	m	sm	m	m	m	m	m	m	m
	%	100	93.4	77.0	70.4	56.3	44.6	32.1	28.1	25.3	25.0	21.9
	C							sm				

r^+ is the ratio of the short arm divided into the long arm. For a ratio of 1.0–1.7 the chromosome type is metacentric (m); 1.7–3.0 is submetacentric (sm); 3.0–7.0 is subtelocentric (st); 7.0 and above is telocentric (t). The positions of the secondary constrictions (C) are based on similar ratios. The chromosome lengths are expressed as a percentage of the longest chromosome in the karyotype.

different from *B. spinolosus*. The karyological differences between both species here reported permit us to confirm this idea from another point of view.

All *Bufo* species examined have 22 chromosomes, except the African *Bufo regularis* species group (2n = 20) and *Bufo* sp. (similar to *B. keringayae*)[13] from Ethiopia, which are diploid-tetraploid species (2n → 4n = 40). The remarkable chromosomic uniformity, specially in number and centromeric index do not permit us to reach systematic and phylogenetic conclusions about the genus, and for this reason the secondary constrictions have been emphasized





Karyotype of A) *B. rubropunctatus*, B) *B. chilensis* (Santiago), C) *B. chilensis* (Los Angeles), D) *B. spinolosus*, E) *B. variegatus*. The line equals 10 µm.

to construct the *Bufo* phylogeny[7]. All the species here analyzed have only 1 secondary constriction in 1 small chromosomic pair (specially pair 7). The karyotypes of the primitive leptodactylid frogs (Telmatobinae)[14-16] have a primitive formula (2n=26)[17] and also show a secondary constriction on pair 7. If the bufonid toads are leptodactylid derivatives, as many authors have considered[18,19], the karyotypes of *Bufo* species may have some similar characters to the leptodactylid frogs. Among them the secondary constriction could be included. The ceratophrynid toads (Ceratophrydae), other leptodactylid derivatives[18], also have 26 chromosomes and also have a secondary constriction on pair 7[20]. The wide distribution of 1 secondary constriction (specially on pair 7) among the karyotypes of the primitive leptodactylid frogs and the leptodactylid derivatives (Bufonidae and Ceratophrydae) suggests the possibility that this character could be considered to be primitive.

Other South American *Bufo* species with this primitive character are: *B. marinus, B. atacamensis, B. paracnemis, B. granulosus, B. poeppi* and *B. crucifer.* The latter species may be the most similar of all living *Bufo* to the ancestral form to the genus[21]. According to Bogart[7], *B. marinus, B. atacamensis, B. paracnemis* and *B. variegatus* could have arisen from a primitive ancestor possessing only K secondary constriction. If the same idea is applied to the species karyologically analyzed *(B. rubropunctatus, B. chilensis* and *B. spinolosus)* then they could also have derived from the same ancestor as *B. marinus, B. atacamensis, B. paracnemis* and *B. variegatus.*

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A view on intramembraneous particles

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Summary. Intramembraneous particles of the outer membrane of *Escherichia coli* show complementary pits on the opposite fracture face. This complementarity characteristic has been discussed in relation to the nature of the particle and the mode of fracturing.

Freeze-fracturing splits membranes into 2 halves, thus allowing an examination of the membrane's interior. The 50-100 Å particles visible on both monolayers are widely assumed to be proteinaceous in nature[2,3].

Most membranes do not reveal complementary impressions (or pits) opposite to particles. Even when it is considered that shadowing, contamination or fracturing itself might obscure complementary pits[4-6], there is no satisfactory explanation why under similar physical circumstances matching halves of other membranes can be visualized. Convincing examples for non-complementarity are the particles of the erythrocyte membrane[4], the purple membrane of Halobacteria[7] and of rhodopsin containing membranes[8]. For the erythrocyte membrane, it is now well established from labelling experiments[9,10] and recombination studies[11,12] that proteins i.e. band III protein and glycophorin represent the particles. These proteins are spanning the membrane[9,13,14]. The number of α-helices penetrating is at least 6 in the native particle. Similarily this has been demonstrated elegantly for the purple membrane[15], which also gives non-complementary fracture faces. In this membrane, about 64-82 α-helices should be involved per particle[16]. A common feature of these membranes is that etching (sublimation of ice) provokes craters in the outer fracture face[8,17]. In these types of membranes, the particle reflects protein; however, shape and mol.wt (tertiary structure) are difficult to assess.

Recently we found that the outer membrane of *Escherichia coli* K12 reveals particles on the outer fracture face (OM i.e. EF) and pits on the inner fracture face (OM i.e. PF)[18,19]. This morphological characteristic has been analyzed in more detail.

Materials and methods. E. coli K12 strain CE 1054 was grown in yeast broth medium as described elsewhere[20]. This strain was used because the fracture plane runs exclusively through the outer membrane. Glycerol was added to prevent freeze damage. The cells were quenched from 23 °C and freeze fractured at −115 °C in a Denton freeze etch apparatus.

Results and discussion. Particles (5000-6000/μm^2) are present on the OM (EF) and pits (4000-5000/μm^2) are observed on the OM (PF) in this membrane. The size of the particles varies between 40-80 Å in diameter. The pits are between 40-60 Å in diameter. It is reasonable to assume that the pits are prints of the particles on the opposite fracture face which means that in this membrane most intramembraneous particles show complementarity upon fracturing.

What might be the biochemical basis of complementarity in these membrane types? As a principle, complementarity