Preparation of yeast cells and bud scars. *S. cerevisiae* was grown and prefixed with glutaraldehyde as described previously[1]. The cells were suspended in either Buffer A or in 0.05 *M* phosphate buffer, pH 6.8 (2 ml, $A_{650\,nm}$ 5.0) that contained 0.2 µmole $CaCl_2$, 0.1 mg BSA and *Arthrobacter* α-mannanase[11] (0.0027 unit[15]). The suspensions were incubated for 20 h at 25 °C. The amount of mannose released in the supernatant corresponded to 12% of the cell's total sugars[16].

Bud scars were prepared from *S. cerevisiae* cell walls by the action of a β-(1→3)glucanase as described previously[5] and suspended in water.

Results and discussion. Intact *S. cerevisiae* cells could not be marked with WGA (Sol I) indicating that WGA receptor sites were not exposed on the cell surface. However, when the cells were treated with the α-mannanase, WGA receptor sites were located on the bud and bud scars but not on the mother cell (Figures 1 and 2). The receptor sites could have been attributed either to chitin, chitin oligomers, the N-acetyl-D-glucosamine link of the mannan-protein or glycolipids containing di-N-acetyl chitobiose which have been found in membrane preparations[17]. The various possibilities were tested against the following informations: Chitin has been shown to be present only in the bud scars[5]. In control experiments, WGA marking was not only totally inhibited by penta-acetyl chitopentaose[18] (2 mg/ml) but also by the mannan-protein (2 mg/ml) prepared enzymatically by action of a β(1→3) glucanase on the cell walls[19] indicating that in solution the WGA marker reacted with the mannan-protein. As WGA is specific for diacetyl chitobiose or higher chitin oligomers[20], this confirmed that the mannan-protein link is a diacetyl chitobiose (or a higher oligomer)[10] and that the protein moiety must lie deep in the cell wall[9] since intact cells were not marked. However, it is doubtful that the WGA receptor sites found on the bud are located in the mannan-protein, since both bud and mother cells were homogeneously marked with ConA (Sol I) which reacts with the side chains of mannan (Figure 3). When the side chains were removed with the α-mannanase, marking was diminished (Figure 4). Glycolipids containing diacetyl chitobiose[17] could not be localized by WGA on *S. cerevisiae* protoplasts, although membrane mannan was well marked with ConA[21]. Therefore the nature of the WGA receptor sites on the bud (Figure 1) is still unknown.

Bud scars prepared enzymatically contain chitin and mannan[5]. They showed WGA receptor sites mainly on the ring structure when examined by transmission electron microscopy (Figure 5). No label was observed when penta-acetyl chitopentaose (2 mg/ml) was present in the labelling mixture (Figure 6). Under the same conditions, marking of mannan with ConA (Sol II) was weaker. Therefore the WGA receptor sites on the bud scars must be attributed to chitin. This is further supported by the fact that chitin synthesis begins at the onset of budding[22] which is shown by the WGA marking at the mother cell-bud junction (Figure 1, double arrows)*.

[15] J. S. TKACZ, J. O. LAMPEN and C. SCHUERCH, Carbohyd. Res. *21*, 465 (1972).
[16] M. DUBOIS, K. A. GILLES, J. K. HALMILTON, P. A. REBERS and F. SMITH, Analyt. Chem. *28*, 350 (1956).
[17] L. LEHLE and W. TANNER, Biochim. biophys. Acta *399*, 364 (1975).
[18] J. A. RUPLEY, Biochim. biophys. Acta *83*, 245 (1964).
[20] I. J. GOLDSTEIN, S. HAMMARSTRÖM and G. SUNBLAD, Biochim. biophys. Acta *405*, 53 (1975).
[19] D. A. BUSH and M. HORISBERGER, J. biol. Chem. *248*, 1318 (1973).
[21] M. HORISBERGER, H. BAUER and J. ROSSET, submitted for publication.
[22] E. CABIB and B. BOWERS, J. Bact. *124*, 1586 (1975).

New Karyological Data of *Rhinoderma*: the Chromosomes of *Rhinoderma rufum*[1]

J. R. FORMAS[2,3]

Instituto de Zoologia, Universidad Austral de Chile, Casilla 567, Valdivia (Chile), 4 February 1976.

Summary. The chromosomes of the Chilean frog *Rhinoderma rufum* are described for the first time. This chromosome set is compared with the karyotype of *R. darwinii*. The importance of the karyological data applied to the phylogeny and systematics of this genus are discussed. A tentative hypothesis of karyological evolution of *Rhinoderma* is given.

Amongst frogs of the superfamily Bufonoidea[4], familiar status and phylogenetic relationships of *Rhinoderma* are controversial[4–6]. The frogs of this genus, endemic of the cool and humid forest of Southern Chile, have a unique life history among the Anuran-tadpoles development in the male vocal sacs. In recent years, karyological data have been an important tool for phylogenetic and systematics studies. From this point of view, some authors[7,8] studied the chromosomes of *Rhinoderma darwinii* and concluded that the genus belongs to the family Leptodactylidae and shows karyological affinities with the Telmatobinae species[7].

Until recently, only one species of *Rhinoderma* was known (*R. darwinii*); however, FORMAS et al.[9] added another species (*R. rufum*) to the genus demostrating the true identity of the enigmatic Chilean frog *Heminectes rufus* Philippi 1902. The 2 species are different in morphology of the feet and developmental patterns.

In this paper, the chromosomes of *R. rufum* are described for the first time. This chromosomal set is compared with the karyotype of *R. darwinii*, which is here redescribed. The importance of the karyological data applied to the phylogeny and systematics of *Rhinoderma* are discussed. A tentative hypothesis of karyological evolution of *Rhinoderma* is given.

The frogs used in this study included: 8 males and 12 females of *R. darwinii* from the vicinity of Valdivia

[1] Supported by Proyecto No. C-7, Vice-Rectoría Investigación Universidad Austral de Chile.
[2] I am grateful to Dr. R. P. SCHLATTER for this comments on the manuscript.
[3] The technical assistence of SONIA LACRAMPE and RAQUEL ULLOA is acknowledged with appreciation.
[4] J. D. LYNCH, in *Evolutionary Biology of the Anurans* (Ed. J. L. VIAL; Univ. Missouri Press, Columbia 1973), p. 173.
[5] I. GRIFFITHS, Proc. zool. Soc., London *132*, 457 (1959).
[6] J. M. CEI, *Batracios de Chile* (Ediciones de la Universidad de Chile, Santiago de Chile 1962).
[7] A. BARRIO and P. RINALDI DE CHIERI, Physis *30*, 673 (1971).
[8] A. VELOSO, N. DÍAZ and R. GALLEGUILLOS, An. Mus. Hist. nat. Valparaíso. *6*, 57 (1973).
[9] J. R. FORMAS, E. PUGIN and B. JORQUERA, Physis, in press.

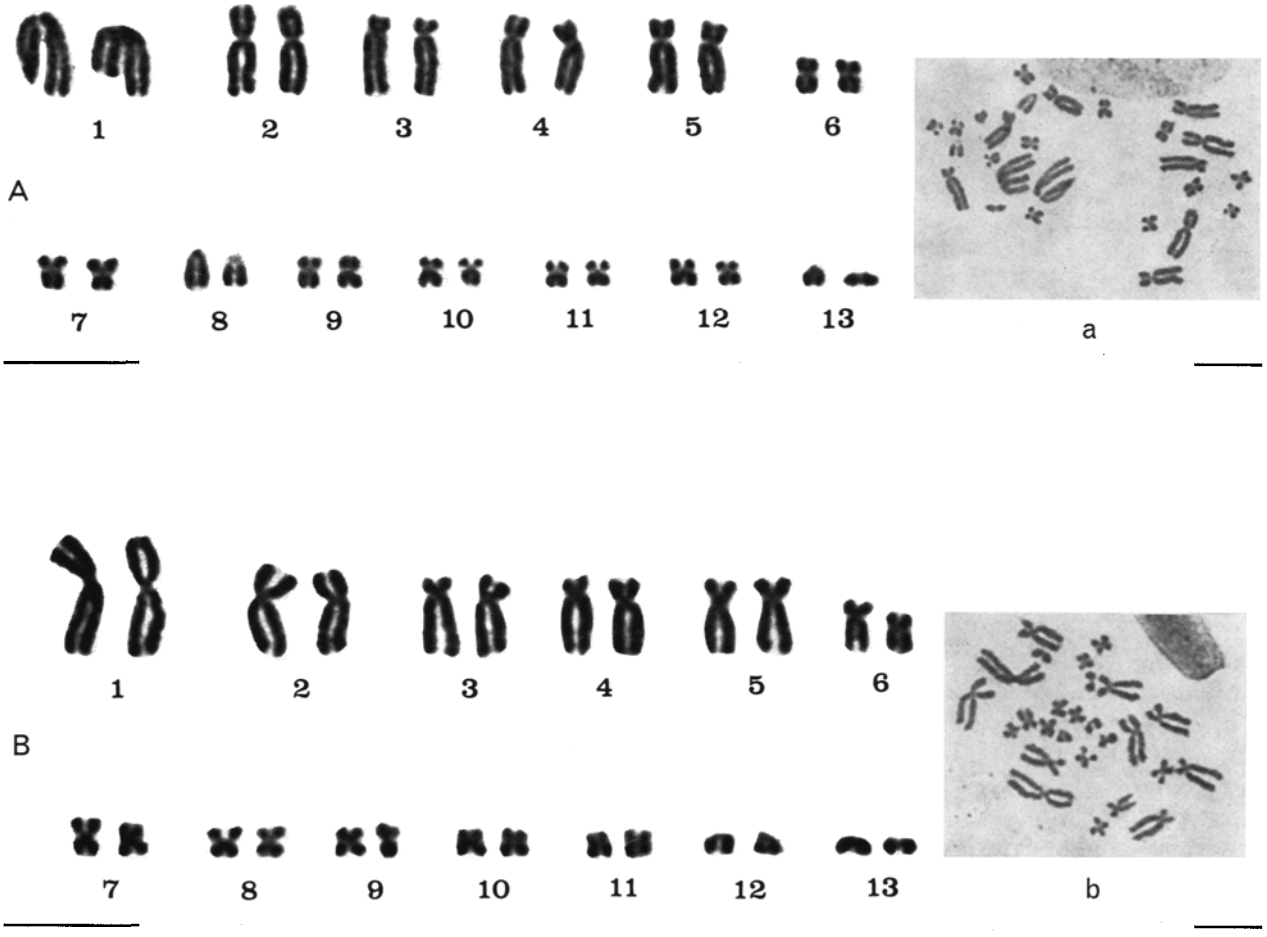
city (Valdivia Province) and 3 males and 14 females of *R. rufum* from Chiguayante (Concepción Province). The specimens karyologically examined have been catalogued in the collection of Amphibians of the Instituto of Zoología of the Universidad Austral (IZUA), in Valdivia. Adults of both species were injected with 0.1% colchicine solution and chromosomes from intestine were obtained (29 mitotic plates *R. darwinii* and 42 mitotic plates *R. rufum*). Fragments of intestine, hypotonically treated, were fixed in acetic-alcohol (1:3) and placed in 45% acetic acid[10]. Small fragments were squashed between 2 slides and stained with acetic orcein. For a more direct comparison of species, the chromosomes were given percentile values with the longest chromosome being 100. Chromosomes more than 50% of the length of the longest (first) are considered large, those from 40–50% intermediate, and those below 40% small. The centromeric position was determined according to LEVAN et al.[11]. Relative length (large, intermediate and small chromosomes) and arm radio (length of the long arm/length of the short arm) of each chromosome were calculated from measurements made on enlarged microphotographs of 5 chromosome figures.

Rhinoderma rufum. The chromosomic set of *R. rufum* (Figure A, a) consists of 26 chromosomes. The fundamental number (FN) is 48. A distinct gap in relative length is evident between Nos. 5 and 6; chromosomes are divided into 2 groups; large chromosomes (Nos 1–5) and small chromosomes group (Nos. 6–13). Pairs 1, 6, 7, 9, 10, 11 and 12 are metacentric (*m*); pairs 2, 4 and 5 are submetacentric (*sm*); pair 3 is subtelocentric (*st*); and pairs 8 and 13 are acrocentric (*t*). Pairs 7 and 10 have subterminal secondary constrictions in the smaller arms. Morphological differentiation of sex chromosomes was not observed in this species.

Rhinoderma darwinii. The formula $2n = 26$ is present in all the mitotic plates. The fundamental number (FN) is 48. The karyotype (Figure B, b) is made up of 5 pairs of large chromosomes (Nos. 1–5) clearly differentiated from 8 small pairs (Nos. 6–13). Pairs 1, 2, 7, 8, 9, 10 and 11 are metacentric (*m*); pairs 3, 4, 5 and 6 are submetacentric (*sm*); and pairs 12 and 13 are acrocentric (*t*). Pairs 7 and 8 have subterminal secondary constrictions in the smaller arms. No sexual chromosomes were observed amongst both *Rhinoderma* species. The results of chromosome measurements and a karyotypic comparison between both species of *Rhinoderma* are included in the Table. As shown here, from a karyological point of view both species of *Rhinoderma* are clearly differentiable.

The frogs of the genus *Rhinoderma* have the same chromosomic formula ($2n = 26$)[12] as many other members of the superfamily Bufonoidea. A karyotype with 26

[10] E. OLMO, Caryologia *25*, 33 (1972).
[11] A. LEVAN, K. FREDGA and A. SANDBERG, Hereditas *52*, 201 (1964).
[12] A. MORESCALCHI, in *Cytotaxonomy and Vertebrate Evolution* (Eds. A. B. CHIARELLI and E. CAPANNA; Academic Press, London 1973), p. 265.



Karyotype (A) and mitotic plate (a) of *R. rufum*; karyotype (B) and mitotic plate of *R. darwinii*. The lines equal 10 μm.

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

Species *Rhinoderma rufum*		Chromosomes												
		1	2	3	4	5	6	7	8	9	10	11	12	13
	r[a]	1.5	1.8	3.2	2.0	2.0	1.2	1.2	∞	1.5	1.6	1.0	1.0	∞
	type	*m*	*sm*	*st*	*sm*	*sm*	*m*	*m*	*t*	*m*	*m*	*m*	*m*	*t*
	%	100	69.3	65.3	63.0	59.6	27.2	26.7	26.4	24.4	22.7	20.4	19.3	17.6
	C							*sm*	*sm*					
R. darwinii	*r*	1.5	1.6	2.7	2.6	2.2	2.3	1.3	1.4	1.6	1.7	1.7	∞	∞
	type	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*	*t*	*t*
	%	100	79.8	65.6	61.2	60.9	36.6	28.4	27.5	26.3	23.6	21.5	19.2	18.0
	C							*sm*			*sm*			

[a] *r* is the ratio of the short arm divided into the long arm. For a ratio of 1.0 to 1.7 the chromosome type is metacentric (*m*; 1.7 to 3.0 is submetacentric (*sm*); 3.0 to 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.

chromosomes, all bi-armed (NF 52)[12], is postulated as basic for the 'higher' Anura (here, only the Acosmanura[13] are considered 'higher' Anura). This formula appeared for the first time among the species of Pelobatidae (*Leptobrachium*[12], *Megophrys*[12], *Pelobates*[12,14], and *Scaphiopus*[15,16]) family to which the bufonoids (Bufonoidea) frogs are probably related[17]. Morescalchi[18] attempted this hypothesis on a karyological basis. The leptodactyloid frogs constitute one of the most extensive (Neotropical Region, Southern South Africa and Australo-Papuan Region) and interesting group of families (Leptodactylidae and Miobatrachidae) of the 'higher' Anura, which the other bufonoid families probably lack. According to REIG[19], the primitive chromosomic number for this group is 26 and I think that the NF should be 52, as Morescalchi[12] postulated as basic for the 'higher' Anura. This primitive formula ($2n = 26$ and NF 52) was maintained in the ancestral stock of leptodactylids, which are represented in South America by the subfamily Telmatobinae[7]. According to our results, it could be alleged that since *Rhinoderma* and many Telmatobinae species share a very similar primitive chromosomic formula ($2n = 26$), they should be closely related. However, this conclusion would be based on a false interpretation of the chromosomic evidence for phylogenetic inference, since the possession of 26 chromosomes is probably a primitive character for the Bufonoidea. According to HENNING[20], the sharing of a primitive character state (symplesiomorphy) does not indicate close phylogenetic relationships; instead this must be inferred from the common possessions of derivated character states (synapomorphy). Therefore the chromosomal data do not afford new relevant evidence that permit us to settle the conflingting views of relationships and familiar status of *Rhinoderma* among the families of Bufonoidea. The results here obtained reinforce the previously known picture of the wide-spread occurrence of the formula $2n = 26$ among the members of Bufonoidea. Robertsonian mechanisms (centric fisions and fusions) have been generally postulated for chromosomic evolution in Anura[21]. Reduction or increment of chromosomic number are characteristic of these karyological changes, while the fundamental number remains constant. However, *Rhinoderma* species maintain the same primitive formula ($2n = 26$), like their leptodactyloid ancesters, but the NF is changed from 52 to 48. The constancy of the chromosomic formula and the change in the NF suggest that the Robertsonian mechanisms have no influence on the karyological evolution of *Rhinoderma* species. The presence of 2 acrocentric chromosome pairs in *Rhinoderma*'s karyotype and conservation of the chromosomic formula ($2n = 26$) suggest that pericentric inversions or other types of translocations could be related to these karyological evolutions.

[13] P. H. STARRET, in *Evolutionary Biology of the Anurans* (Ed. J. L. VIAL; Univ. Missouri Press, Columbia 1973), p. 251.
[14] A. MORESCALCHI, Rc. Acad. Sci. fis. mat., Napoli *31*, 326 (1964).
[15] A. O. WASSERMAN and J. P. BOGART, Copeia *2*, 303 (1968).
[16] A. MORESCALCHI, Experientia *24*, 964 (1968).
[17] J. D. LYNCH, Misc. Publs Mus. nat. Hist. Univ. Kansas *53*, 1 (1971).
[18] A. MORESCALCHI, Experientia *23*, 1071 (1967).
[19] O. A. REIG, in *Evolution in the Genus Bufo* (Ed. W. F. BLAIR; University of Texas Press, Austin and London 1972), p. 34.
[20] W. HENNING, *Elementos de una sistemática filogenética* (Editorial Universitaria, Buenos Aires 1968).
[21] M. L. BEÇAK, Caryologia *21*, 191 (1968).

Thermodynamic Aspects of Development for *Tenebrio molitor* L.

K.-D. LOEHR, P. SAYYADI and I. LAMPRECHT

Zentralinstitut für Biochemie und Biophysik der Freien Universität Berlin, Habelschwerdter Allee 30, D-1 Berlin 33 (German Federal Republic, BRD), 16 February 1976.

Summary. Predictions of the thermodynamics of irreversible processes are tested for the development and aging of an insect. Specific heat production and specific respiration rate decrease towards a steady state with deviations for the time of hatching of the imago.

It has long been known that classical thermodynamics does not apply to living matter. The concept of the evolution towards minimum free energy and maximum entropy is bound to closed systems, while organisms per se are open systems exchanging energy and entropy with their surroundings. The attempt to prove the theory of linear irreversible processes in this field could only be a zero order approach, since animals are normally far from equilibrium, and the linear relationships between flows and forces are only valid near equilibrium [1–3]. Therefore,