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Karyotype of a gekkonid lizard, *Eublepharis kuroiwae kuroiwae*[1]

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Summary. Eublepharis kuroiwae kuroiwae, a morphologically primitive gecko endemic to the Ryukyu Archipelago, Japan, has 2n = 24 chromosomes, with 7 large biarmed, and 1 large and 4 small uniarmed homologous pairs. This is the smallest chromosome number so far described in the family Gekkonidae. The presence of two distinct size groups, and the numerical dominance of metacentric or submetacentric pairs are also characteristic features.
Key words. Eublepharis k. kuroiwae; Reptilia; Gekkonidae; karyotype.

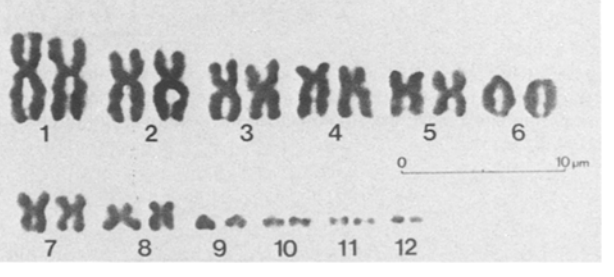
Kluge[2] divided the family Gekkonidae into four subfamilies, of which the Eublepharinae is regarded as the most primitive stock on the basis of its morphological features. Although intensive karyological studies have recently been performed upon members of other subfamilies[3–10], little information exists about the karyotypes of eublepharine lizards despite their possible great significance for the understanding of gekkonid evolution[11,12]. We have performed the first examination of the chromosomes of *Eublepharis kuroiwae kuroiwae,* a member of the Eublepharinae endemic to the Ryukyu Archipelago, Japan. Our results show this species to be karyologically highly specialized with a chromosome morphology different from those of other gekkonids, including three eublepharine species hitherto karyotyped.

Materials and methods. Two males and six females of *E.k.kuroiwae* were collected from Okinawa Island, Ryukyu Archipelago, in September, 1986. They were injected i.p. with 0.1 ml of colchicine solution (2 mg/ml) per g b.wt, 20 h before being sacrificed. The cells from femur bone marrows were treated with hypotonic KCl (0.06 M) solution for circa 30 min, followed by fixation in glacial acetic acid-absolute methyl alcohol (3:1). Mitotic chromosome preparations were made by an air-dry method and stained in 20% Giemsa solution. Chromosome description follows the terminology of Green et al.[13].

Results. The chromosome number was determined as 2n = 24 on the basis of 62 well-spread metaphase cells. No sex chromosome heteromorphism was evident. Chromosome pairs 1–8 were distinctly larger than pairs 9–12. Of the larger group, only pair 6 was acrocentric, and the others were biarmed; pairs 1, 3, 5 and 7 were regarded as metacentric, and pairs 2, 4 and 8 as submetacentric. All the elements belonging to the smaller-sized group were acrocentric (fig.). Therefore, the fundamental number (NF) was calculated as 38.

Discussion. The family Gekkonidae consists of approximately 650 species[14], of which only about 50 have hitherto been karyotyped. The known diploid number of gekkonids ranges from 24 to 46[12,14–16], and the diploid count of 24 reported here for *E.k.kuroiwae,* equivalent to that for *Anarbylus switaki*[12], is the smallest number recorded for this family. Several authors have assumed the typical gekkonid karyotype to be characterized by having many uniarmed and few biarmed elements, which do not form distinct size groups[11,14,17]. Therefore, the karyotype of the morphologically primitive *E.k.kuroiwae* is regarded as representing a highly specialized condition within the family, and exemplifies the inconsistency of morphological and karyological derivations.

Of approximately 20 species belonging to the subfamily Eublepharinae, karyological data are available only for *Eublepharis macularis* from Southwest Asia, and *Coleonyx variegata* and *Anarbylus switaki* from North America. They have graded series of chromosomes, 2n = 38 (all acrocentric; NF = 38), 32 (all acrocentric; NF = 32), and 24 (22 metacentric and 2 acrocentric; NF = 46) in number, respectively[11,12]. Although *E.k.kuroiwae* more closely resembles *A.switaki* in terms of chromosome counts and morphology of larger elements, the fact that *E.k.kuroiwae* and *E.macularis* have identical NF values seems to indicate their closer



The karyotype of female *Eublepharis kuroiwae kuroiwae.*

karyotypic relationship. King[17] assumed fusion to be the major mode of karyological differentiation in lizards. The highly specialized karyotype of *E.k.kuroiwae* appears to have derived from the *E.macularis* type through Robertsonian fusions. Further karyological analyses with banding techniques on these two species, and other congeners occurring in intermediate regions[18], are required to test our hypothesis.

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Sperm chymotrypsin-like enzymes of different inhibitor-susceptibility as lysins in ascidians

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Summary. Inhibitory effects of three peptidyl phenylalaninals on fertilization and on chymotrypsin-like enzyme activity of sperm in three species of ascidians were examined. The results suggest that a sperm chymotrypsin-like enzyme is indispensable for the fertilization in each of the ascidians, and that these enzymes have different susceptibilities to inhibitors.
Key words. Fertilization; sperm; chymotrypsin; lysin; ascidian.

It is currently known that spermatozoa penetrate through egg investments with the aid of sperm-bound lytic agents, the lysins. In ascidians, which occupy a phylogenetic position between vertebrates and 'true' invertebrates, sperm proteases are thought to function as lysins, similar to those of mammals[2].

In our previous study of the sperm lysin system of the ascidian, *Halocynthia roretzi,* we reported that two trypsin-like enzymes, acrosin and spermosin, and a chymotrypsin-like enzyme are indispensable for sperm penetration through the vitelline coat of eggs[3–7].

Furthermore, the timing of action of these three proteases in the fertilization of *H. roretzi* has also been demonstrated[8]. In the present report, we attempt to investigate the lysin system of other ascidians and compare the inhibitory effects of three microbial chymotrypsin inhibitors on the fertilization and on the sperm chymotrypsin activity in each of the ascidians.

Materials and methods. Gametes of *H. roretzi* from Mutsu Bay were collected from a pair of gonads with gonaducts as described previously[4], while those of *Ciona savignyi* and *Ascidia ahodori* from Mutsu Bay were collected from the gonaducts with a Pasteur pipette. Eggs (100–200) were incubated for 1–2 min in 1 ml of seawater, filtered and buffered with 10 mM Tris-HCl (pH 8.0), containing various concentrations of protease inhibitors, and then inseminated at the temperature of seawater at which each ascidian spawns, i.e., 13 °C for *H. roretzi,* 15 °C for *C. savignyi,* or 21 °C for *A. ahodori.* The percentage of fertilization in *H. roretzi* was determined at 30 min after insemination on the basis of the expansion of the perivitelline space and again at 2 h on the basis of the first cleavage. Eggs which had undergone either of these reactions were counted as fertilized ones. Fertilization ratios in *C. savignyi* and *A. ahodori* were determined at 2 h and 1 h, respectively, after insemination, on the basis of the first cleavage, since the expansion of the perivitelline space following insemination was not observed in these ascidians.

Spermatozoa stored at −40 °C were thawed, homogenized in 10 vols of artificial seawater (460 mM NaCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, and 10 mM KCl), buffered with 10 mM Tris-HCl (pH 8.0) using a Teflon homogenizer (1000 rpm, 10 strokes), and stirred for 2 h. After centrifugation (10,000 × g, 30 min), the resulting supernatant was used as an enzyme extract. Chymotrypsin and trypsin activities of the sperm extract were assayed fluorometrically (excitation at 380 nm, emission at 460 nm) at 25 °C in 50 mM Tris-HCl (pH 8.5) containing 10 mM $CaCl_2$ and 0.1 mM bestatin using succinyl (Suc)-Leu-Leu-Val-Tyr-4-methylcoumaryl-7-amide (MCA) (Peptide Institute) and t-butyloxycarbonyl (Boc)-Val-Pro-Arg-MCA (Peptide Institute) as substrates for chymotrypsin- and trypsin-like enzymes, respectively. Inhibition of chymotrypsin activity by three microbial chymotrypsin inhibitors was determined by measuring the residual activity after prior incubation with the inhibitors for 30 min. Chymostatin was purchased from the Peptide Institute (Osaka). Leupeptin and bestatin were generous gifts of Dr W. Tanaka of Nippon Kayaku Co. α- and β-MAPI were prepared as described previously[9].

Results and discussion. Hoshi has described that only the chymotrypsin-like enzyme of spermatozoa seems to be responsible for sperm penetration through egg investments in one order of the ascidians, the Enterogona, whose gonad is unpaired and lies within or behind the loop of the intestine. On the other hand, both chymotrypsin-like and trypsin-like enzymes are required in the other order, the Pleurogona, in which a pair of gonads are in the lateral mantle wall[2]. In *H. roretzi* of the Pleurogona, the fertilization has been demonstrated to be inhibited with chymostatin, a chymotrypsin inhibitor, and leupeptin, a trypsin inhibitor[3]. In this study, we investigated the susceptibility of fertilization to the inhibitors quantitatively, comparing *H. roretzi* (Pleurogona) with *C. savignyi* and *A. ahodori* (Enterogona). The results of the comparison are shown in figure 1. As described previously[3], the fertilization of *H. roretzi* was completely inhibited not only with chymostatin but also with leupeptin, though the effective concentration of the latter was approximately 3-fold higher than that of the former. On the other hand, the fertilization of *C. savignyi* and *A. ahodori* was inhibited only with chymostatin. Leupeptin scarcely inhibited, even at a