structed plasmids yielded recombinant polypeptides presenting very different immunogenicity. We have performed the Chou and Fasman prediction of some of their most representative (high or low immunogenicity) constructs (those named 1, 3, 4 and 10 by these authors). The figure shows the prediction of the fusion polypeptide N-terminus compared with the structure prediction of the HBsAg protein. The corresponding immunogenicities reported by these authors are also indicated. As can be seen in the figure, the long stretch of β-sheet in construct 'C' alters the fusion protein and results in low immunogenic response, most likely by hindrance caused on the next HBsAg peptide. Construct 'E' shows that the long N-terminal stretch of α-helix in the fusion polypeptide continues into the HBsAg peptide, which also has low immunogenicity.

Another example is the cloning of Foot and Mouth viral antigenic peptide (142–160) as the N-terminus of a fusion polypeptide with residues 9-1023 of β-galactosidase[10]. In this case, the modification would occur in the β-galactosidase peptide, whose junction region changes from a β-sheet to a turn (prediction not shown).

The above considerations can also be extended to protein engineering. At present, it is possible to envisage the design of new proteins via fusion of two or more polypeptides, or domains. In all cases in which new combinations of DNA sequences corresponding to pre-existent protein structures are produced, problems like those discussed above may appear.

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Glischropus tylopus, the first known old-world bat with an X-autosome translocation

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Summary. Glischropus tylopus shows an X-autosome translocation in which a small acrocentric chromosome has been transferred to the X. The diploid number is 30 for the females and 31 for the males. RBG banding shows that in the late replicating X of the female only the original X replicates late, the autosomal part replicates early, showing the same pattern as the corresponding autosomal 'Y_2' of the male. In the X chromosome, a heterochromatic band separates the autosomal from the gonosomal sequences.

Key words. Vespertilionidae; X-autosome translocation; late replicating X; spreading effect.

The genus *Glischropus* (family Vespertilionidae), which is considered to be an offshoot of the widespread genus *Pipistrellus*[3], consists of only two species, i.e. *G. javanicus*, which occurs in Java and has not been karyologically investigated, and *G. tylopus*, occurring in the other parts of South East Asia, which will be treated here.

Glischropus tylopus shows as an interesting morphological specialization white or pink colored pads on thumb and feet, which are considered to be useful for climbing in hollow tubes, e.g. bamboo[4], as is proven for *Tylonycteris*[5] bats (which show, however, differently shaped pads).

Five specimens of *Glischropus tylopus* were karyologically examined, all captured near the University of Malaya Field Study Centre (3°20'N, 101°45'E), Ulu Gombak, Selangor, Malaysia in 1984. Conventionally stained preparations from lymphocyte culture were obtained from three animals (2 males, one female). Fibroblast cultures of heart and lung tissue were established from one male and one female. These slides were used for duplicate staining (AgNOR[6] or CBG[7] banding after quinacrine[8]) or GTG[9] banding. RBG bands[10] were obtained after a 10-h block of thymidine followed by an 8-h BrdU treatment[11].

Results. The karyotype consists of 30 chromosomes in the females and 31 chromosomes in the males. There are 8 large metacentric to submetacentric, two small subtelocentric and 4 acrocentric autosomal pairs. The X chromosome is a large submetacentric one, the two unmatching chromosomes of the males are small acrocentric chromosomes of differing size. After G banding (fig. 1) each pair can be unequivocally identified. The smaller of the two unmatching acrocentric chromosomes of the male shows both the same size and the same faint staining as the short arm of the X chromosome (fig. 1). Therefore it will be regarded as Y_2 which is of autosomal origin. After NOR staining one submetacentric pair shows an NOR in the short arm close to the centromere (see arrow in fig. 1). The centromeres are only faintly stained after C banding, but a broad band of constitutive heterochromatin can be seen in the short arm of the X close to the centromere, thus separating the gonosomal from the autosomal genes (fig. 3). Additionally, the larger of the two unmatching chromosomes of the male is, except for a very narrow distal region, completely heterochromatic. This chromosome is therefore regarded as Y_1, representing the original Y chromosome.

This assignment is confirmed by the results of RBG banding after BrdU incorporation (fig. 2). The X chromosome of the male and the early replicating one in the female show several early replicating bands in the long arm and a broad early

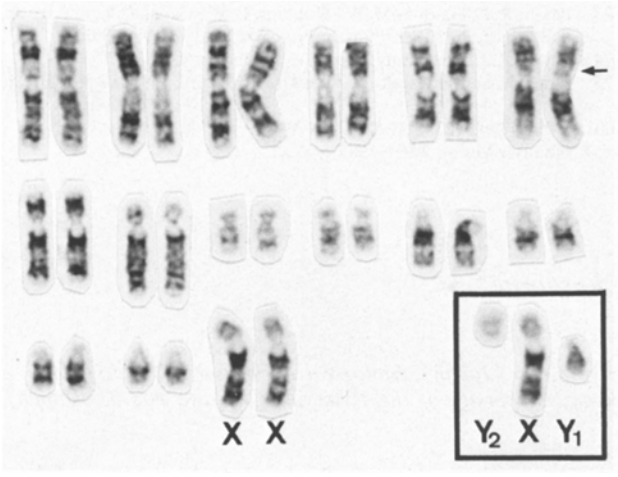

Figure 1. G-banded karyotype of a female *Glischropus tylopus*. The arrow indicates the location of the nucleolus organizer region. The inset shows the sex chromosomes of a male.

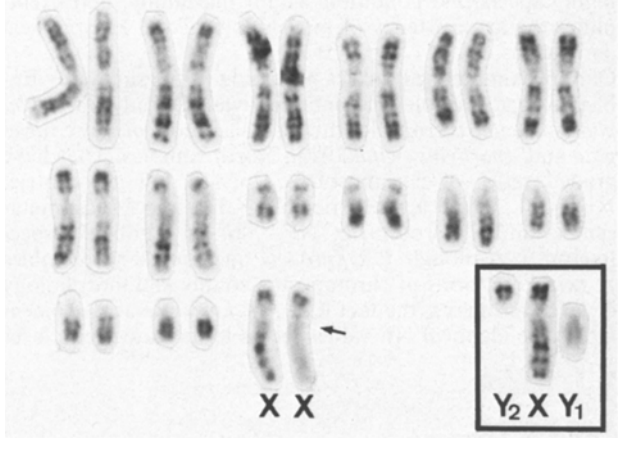

Figure 2. RBG bands of a female *Glischropus tylopus*. The autosomal part of the late replicating X (arrowed) replicates early. Bottom right, the sex chromosomes of a male: the Y_2 shows the same pattern as the autosomal part of the X.

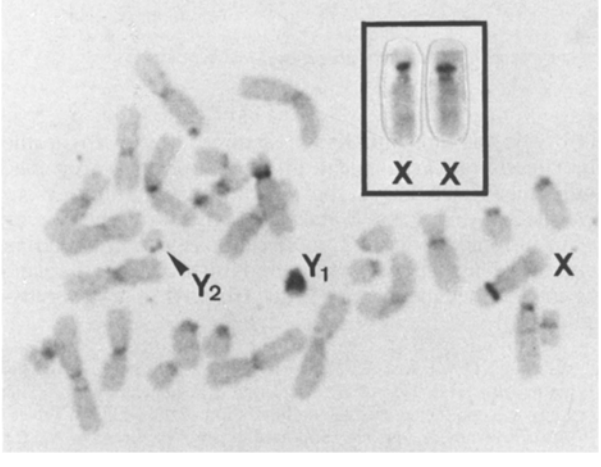

Figure 3. C-banded metaphase of a male *G. tylopus*. The sex chromosomes of a female are shown in the inset. The heterochromatic band in the short arm of the X is clearly separated from the centromeric dots.

replicating region in the short arm. This region consists of two bands and is separated from the centromere by a small late replicating band, which consists of constitutive heterochromatin, according to C banding. In the late replicating X of the female only the long arm replicates late; the short arm shows the same pattern as in the early replicating X. In the male, the heterochromatic Y_1 replicates late; the Y_2, however, as a whole replicates early. It lacks the late replicating region, which in the short arm of the X is situated close to the centromere. Consequently, it possesses only centromeric heterochromatin.

Discussion. Glischropus tylopus shows an X-autosome translocation in which a small acrocentric chromosome has been transferred to an X which in its ancestral form probably was acrocentric. In the resulting submetacentric X a heterochromatic band separates the autosomal from the gonosomal region. In the female, the autosomal parts of both X replicate early. According to Kasahara and Dutrillaux[17] the spreading effect of X inactivation may be blocked by heterochromatic segments. In several cases such a heterochromatic segment has been found in X-autosome translocations: in *Lagorchestes conspicillatus*[12] (Marsupialia), *Taterillus gracilis*[13] (Rodentia), *Tragelaphus imberbis*[14] and *Muntiacus muntjak*[15,16] (Artiodactyla). Surprisingly, none of these species shows any further interstitial or terminal heterochromatin. On the other hand, there are also cases of X-autosome translocations without heterochromatic separation: some species of *Gazella*[18] (Artiodactyla), the rodent *Deltamys kempi*[19] and some bat species of the Carolliinae[20,21] and Stenodermatinae[22] (Phyllostomidae). However, in one of these species, *Artibeus lituratus*, an additional heterochromatic segment has been found in the X[17].

To sum up, a heterochromatic band may be useful for preventing the spreading effect but is not absolutely necessary. Clearly, there must be a 'sign' in each case which prevents inactivation of the autosomal part of the late replicating X. In different mammalian orders species possessing an X-autosome translocation have been discovered, but the advantage of and the reason for such rearrangements are not yet known.

Within the chiropteran order only members of two subfamilies of the Phyllostomidae are known to have X-autosome translocations[23,24]. The occurrence of an X-autosome translocation in the karyologically well examined[25] vespertilionid family is thus noteworthy.

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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.

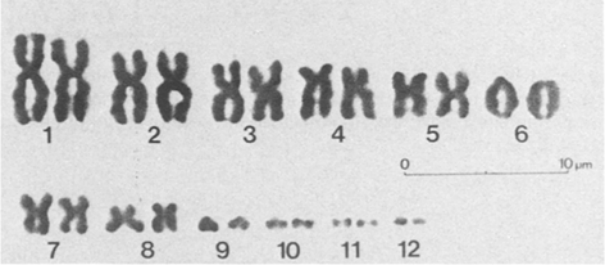
Kluge[2] divided the family Gekkonidae into four subfamilies, of which the Eublepharinae is regarded as the most primitive stock on the basis of its morphological features. Although intensive karyological studies have recently been performed upon members of other subfamilies[3–10], little information 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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