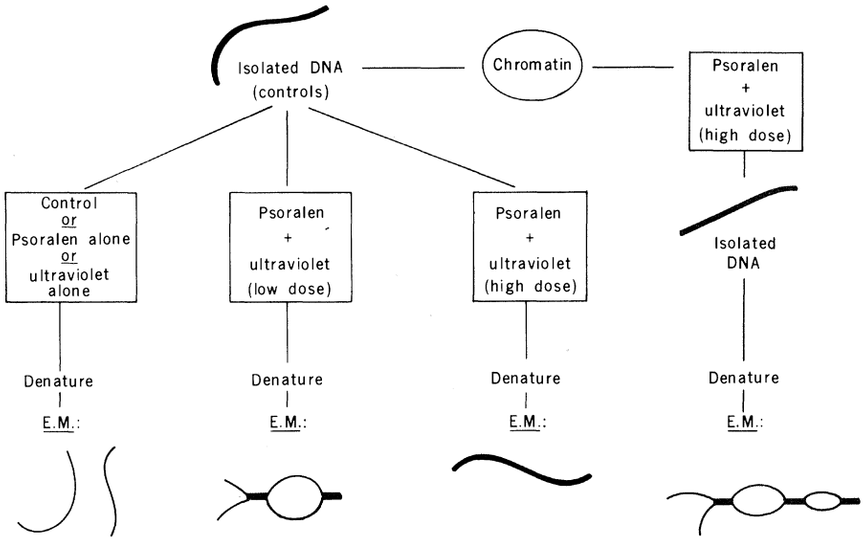

Fig. 3. Experimental design. Before or after photoreaction with trioxsalen, DNA is isolated from chromatin (or intact nuclei or cells) by incubation at 50°C for 5 to 12 hours with 1 percent sarkosyl, 0.1*M* EDTA, 0.01*M* tris, *p*H 8.6, and nuclease-free Pronase (1 mg/ml). The DNA is then banded by equilibrium sedimentation in a gradient of Cs_2SO_4 and dialyzed exhaustively against 0.01*M* tris, *p*H 7.6, 0.001*M* EDTA prior to preparation for electron microscopy as described in the text.

Histograms of the (center-to-center) spacing of loops are more complex. These data are also more difficult to interpret because of the problem of calibrating the length of interloop regions of DNA. These denatured regions have the appearance of native DNA only because of frequent cross-links.

We have observed an essentially similar pattern of alternating cross-linked and protected regions in many systems, including interphase and metaphase HeLa cells, cultured *Drosophila* cells, nuclei isolated from *Drosophila* embryos, isolated HeLa metaphase chromosomes, replicating chromosomes of SV40 virus in vivo, and isolated SV40 chromosomes in vitro.

The possible perturbation of chromatin structure by the psoralen cross-linking probe itself is unknown. However, photoreaction with trioxsalen does not alter the pattern of DNA fragment lengths obtained by subsequent micrococcal nuclease digestion of nuclei (*17*).

An example of subchromosomal variations in cross-linking pattern is found in *Drosophila* nuclei. Fractionation of *Drosophila* satellite DNA by density gradient techniques (*18*) is still possible after cross-linking in situ. The several satellites show loop patterns different from that of main-band DNA and different from those of each other. We believe that these differences result from chromatin structure in centric heterochromatin where these satellite DNA's are localized (*19*).

In conclusion, the ability of the psoralen probe to preserve a linear record of its interaction sites appears to present unique potentials in the study of chromatin structure.

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Karyotype Conservation and Difference in DNA Amount in Anguilloid Fishes

Abstract. *The two Pacific anguilloid fishes* Anguilla japonica *and* Astroconger myriaster, *belonging to the different families, appear to have identical chromosome numbers (2n = 38) and karyotypes, including one pair of conspicuous heteromorphic chromosomes in females. Cytophotometric measurements, however, indicate a considerable difference in DNA content between the two species.*

Considerably different genome sizes (DNA content) have been reported in the divergent fish orders (*1, 2*). In this connection, Ohno (*1*) suggested that quantum evolution from fish to mammal was not accomplished simply by point mutations of already existing gene loci, but rather through duplicated genes which made possible the emergence of new genes with previously nonexistent functions. It is also believed that the primitive teleostean ancestor had approxi-

mately 48 acrocentric chromosomes (*3*). More diverse teleosts arose from this ancestor through gene duplication. In some instances, this did not accompany a change in number and shape of chromosomes. In others, the mechanisms of pericentric inversions, Robertsonian translocations, deletions, and polyploidy were responsible for an increase or a decrease in chromosome number and size (*1*). The karyotypes of anguilloids are little known; only two Atlantic species of the eel family Anguillidae have been analyzed (*4, 5*). Both of them have identical chromosome number ($2n = 38$) and similar heteromorphic pair. Our study establishes the relation between karyotypes and genome size of the two Pacific anguilloid species *Anguilla japonica* and *Astroconger myriaster*, which belong to the families Anguillidae and Congridae, respectively. We used only female specimens. The sex of the specimen was determined by gonadal histology. Karyotypes are based on air-dryings of kidney cells and cultured leukocytes (*6*) or renal cells (*7*).

Upon comparing the karyotypes of two species (Fig. 1), we were impressed by their apparent similarities in spite of phylogenetic distance, that is, belonging to different families. The diploid chromosome numbers of both species were found to be 38, of which 20 were metacentric-submetacentric and 18 were acrocentric chromosomes. Furthermore, both anguilloids contained one pair of chromosomes that did not match. This mismatched heteromorphic pair (ZW?) was made of the largest and the smallest metacentrics. The apparently similar heteromorphic pair was also found in females of the American eel, *Anguilla rostrata* (*4*), as well as in an unspecified sex of European eel, *Anguilla anguilla* (*5*).

Apart from revealing the extremely conservative trend in karyotypic evolution within Anguilliformes, our study suggests an apparent paradox with respect to the genome size. The ratio of nuclear DNA in the Japanese eel to that in the conger eel, as measured by the two-wavelength method (*8*), was 2 to 3, that is, 304 and 426 arbitrary units, respectively. The sizes of cell and nucleus of erythrocytes in conger eels were also markedly larger than corresponding cells of Japanese eels (Table 1).

Table 1. Comparison of nuclear DNA content and erythrocyte size in females from two species of Anguilliformes. Each value represents the mean of five specimens. The relative DNA content was measured from 100 nuclei of the Feulgen-stained hepatocytes in each species (Olympus microspectrophotometer). The relative cellular and nuclear sizes of 100 cells of each species were determined from erythrocyte smears stained with Giemsa. Results are given in arbitrary units (A.U.) ± standard deviation (S.D.).

Species	DNA content (A.U. ± S.D.)	Erythrocyte size (A.U. ± S.D.)	
		Cell	Nucleus
Anguillidae: *Anguilla japonica*	304 ± 29	818 ± 88	190 ± 30
Congridae: *Astroconger myriaster*	426 ± 45	1170 ± 144	280 ± 48

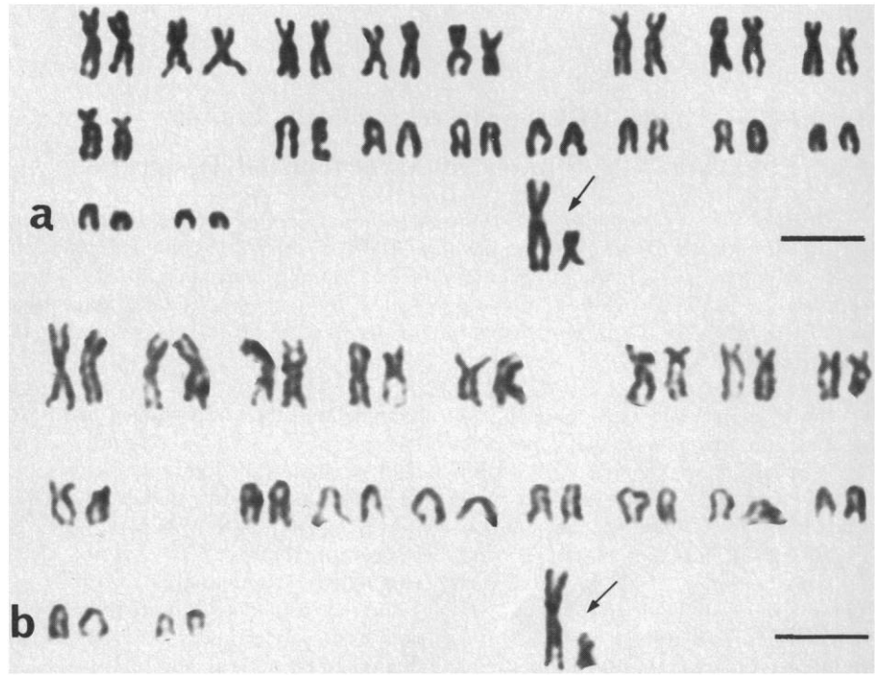

Fig. 1. Karyotypes of females from Japanese eel (a) and conger eel (b) showing the karyotype conservation, including a conspicuous heteromorphic pair (arrow). The analyses comprised 82 and 34 karyotypes among 685 and 118 countable metaphases from 13 specimens of Japanese eel and six specimens of conger eel, respectively. Bars, 5 μm.

Most fishes have morphologically undifferentiated sex chromosome, and the genetic difference between the X and Y or the Z and the W is usually very small (*9*). Under this circumstance, polyploidy may not invariably disturb the sex determining mechanism; that is, it is not incompatible with fertility. Thus, polyploidy is an important evolutionary mechanism in some groups of fishes such as in the orders Cypriniformes (*10*) and Isospondyli (*11*). However, heteromorphic sex chromosomes of the XX-XY, XX-XO, and ZW-ZZ types (*12*), as well as multiple sex chromosomes (*13*), have been described in certain groups of fishes. In contrast to genic control, this well-established chromosomal sex determining mechanism may negate the possibility of polyploid evolution as it does in higher vertebrates. From this point of view, the presence of a heteromorphic chromosome pair which may reflect differentiated sex chromosome of the ZW type in anguilloid fishes may be one of the reasons why their karyotype evolution has been extremely conservative.

There is abundant evidence from isozyme studies in both fishes (*14*), and in other vertebrates (*15*), that gene duplication occurred. The discovery of different genome size within the confines of a conserved anguilloid karyotype suggests that gene duplication, if it occurred, was strictly regional (tandem duplication) in Anguilliformes.

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(+)-α-(*N*-1-Phenethyl)Urea Stereospecifically Inhibits Ca²⁺- but Not ADP-Stimulated Mitochondrial Respiration

Abstract. *The (+) isomer of α-(N-1-phenethyl)urea is a moderately potent inhibitor of Ca^{2+}-stimulated mitochondrial respiration of $^{45}Ca^{2+}$ uptake (50 percent inhibition at ~0.18 mM) while having no effect on adenosine diphosphate–stimulated respiration; the (−) isomer is without effect up to 4 mM. β-Phenethylurea does not inhibit with either stimulus. The data support the involvement of a Ca^{2+}-specific protein in energized mitochondrial Ca^{2+} uptake.*

The hypoglycemic agent phenethylbiguanide, an organic base that is positively charged at physiologic *p*H, competitively inhibits nonenergized mitochondrial Ca^{2+} binding and blocks energized Ca^{2+} uptake, exactly in parallel with inhibition of adenosine diphosphate (ADP)–stimulated respiration (*1*). We therefore explored the structure-function relationships of the phenethylbiguanide molecule, in particular to assess the role of the positive charge as compared to that of the hydrophobic moiety. In the course of our studies, we have found that (+)-α-(*N*-1-phenethyl)urea is a potent inhibitor of Ca^{2+}- but not ADP-stimulated mitochondrial respiration, while the (−) isomer is entirely without activity toward either stimulus. The high degree of structural specificity of this pair of compounds is further emphasized by the observation that β-phenethylurea is completely inactive, while *n*-alkylureas of medium chain length and the homologous monoguanidinium compounds are moderately effective inhibitors of both ADP- and Ca^{2+}-stimulated respiration.

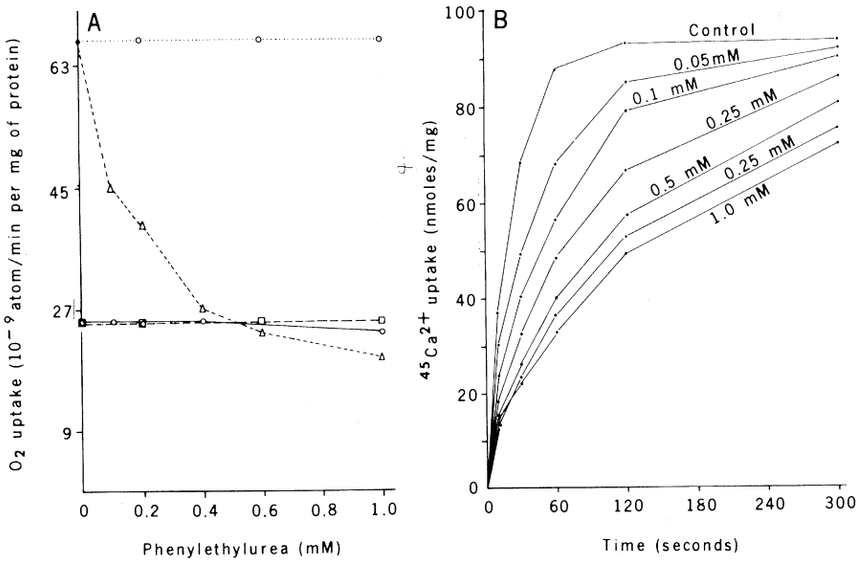

Fig. 1. Inhibition of mitochondrial Ca^{2+}-linked functions by α-(*N*-1-phenethyl)ureas. (A) Oxygen uptake rates. Incubation medium for studies of O_2 uptake contained 0.25*M* sucrose, 10 m*M* HEPES (sodium salt) buffer (*p*H 7.4), and 10 m*M* glutamate. Mitochondrial protein concentration was 4.5 mg/ml, and respiration was initiated with either 0.25 μmole of ADP plus 0.25 μmole sodium potassium phosphate, or 0.5 μmole of Ca^{2+}, in a final volume of 1.6 ml at 30°C. Uptake rate of O_2 was calculated as the stimulated rate minus the state 2 rate (*13*). (△---△), (+)-α-(*N*-1-phenethyl)urea, Ca^{2+}; (○···○), (−)-α-(*N*-1-phenethyl)urea, Ca^{2+}; (○——○), (+)-α-(*N*-1-phenethyl)urea, ADP; and (□---□), (−)-α-(*N*-1-phenethyl)urea, ADP. (B) Rate of $^{45}Ca^{2+}$ uptake. The suspension contained 1.0 mg of mitochondrial protein per milliliter, and uptake was initiated with 0.1 m*M* $^{45}Ca^{2+}$. Concentrations of (+)-α-(*N*-1-phenethyl)urea are indicated on curves.

Guinea pig liver mitochondria were prepared and O_2 uptake was measured as described (*1*); $^{45}Ca^{2+}$ uptake was analyzed by a rapid filtration method (*2*). The (+)- and (−)-α-(*N*-1-phenethyl)ureas (K and K Laboratories) were dissolved in a mixture of ethanol and water without heating and recrystallized twice. Both compounds moved as single components on thin-layer chromatography (TLC) in a butanol, acetic acid, H_2O system. Specific rotations $[\alpha]_D^{24°}$ of the two compounds were +43.6° and −44.6°, respectively. Gas-liquid chromatography revealed the presence of two minor peaks of low molecular weight that were identical in the two stereoisomers; these peaks constituted less than 6 percent of the total mass of each isomer. Mass spectroscopy of each major and minor component revealed no differences between the stereoisomers (*3*). *n*-Alkylureas were obtained from commercial sources. β-Phenethylurea was synthesized as described (*4*); the twice-crystallized product melted sharply at 112°C and moved as a single component on TLC. β-Phenethylguanidine was synthesized as described (*5*). The (+)- and (−)-α-(*N*-1-phenethyl)guanidines were provided by R. Fielden (*6*).

In an experiment showing the effect of (+)- and (−)-α-(*N*-1-phenethyl)urea on Ca^{2+}- and ADP-stimulated respiration (Fig. 1A), 50 percent inhibition of Ca^{2+}-stimulated respiration by the (+) isomer was seen at 0.28 m*M* Ca^{2+}, but the concentration required varied somewhat from experiment to experiment around a mean of about 0.175 m*M* (Table 1). The complete lack of effect of the (+) isomer on ADP-stimulated respiration and the total inactivity of the (−) isomer are also easily apparent. The maximal rate of O_2 uptake with saturating concentrations of ADP (more than 0.25 m*M*) was less than the rate obtained with Ca^{2+}; the (+) isomer differentially inhibited Ca^{2+} but not ADP-stimulated respiration at these maximally stimulated rates. β-Phenethylurea and *N*-benzylurea were entirely without effect on either Ca^{2+}- or ADP-stimulated respiration. In contrast, both the (+) and (−) isomers of α-(*N*-1-phenethyl)guanidine were moderately potent respiratory inhibitors, slightly more effective with ADP than with Ca^{2+} as the respiratory