spectively) zones were identified and cut away. The remaining material was eluted with 0.05*N* formic acid and lyophilized. The dried material was taken up in 5 ml of 0.05*N* formic acid and chromatographed on Sephadex G-25 (column, 5 by 83 cm). Five fractions or peaks were identified (Fig. 1). There was evidence for peptide throughout the chromatogram from the absorbance at 230 nm and especially in fraction 2, where the major 230-nm peak corresponded with the ninhydrin-positive peak.

Molecular sieving of the biologically active ninhydrin fraction 2 indicates that its molecular size is between 1000 to 1500 daltons. Two and sometimes three 260-nm absorption peaks eluted later and were ninhydrin-negative. When these fractions were tested in the cyclic AMP–dependent protein kinase assay, inhibitory activity appeared in fractions 1 to 3; the major activity was in fraction 2, which also was the only fraction with increased inhibitory action of insulin, as compared to the control, whether the assay was performed in the presence or absence of cyclic AMP (Table 1).

Fractions 1 and 2 extracted from insulin-treated muscle also activated muscle phosphoprotein phosphatase in a dose-dependent manner, with fraction 2 being more potent (Fig. 2). At higher concentrations, fraction 2 had an inhibitory effect on the phosphatase. This effect may be due to the impure nature of the material. Fraction 3 was very weakly inhibitory. Further purification of fraction 2 by thin-layer chromatography (cellulose; ammonium acetate developer, and ethanol, *p*H 3.8) produced six fractions, which were assayed. Fraction 2d, which contained the inhibitor of the cyclic AMP–dependent protein kinase, demonstrated the difference between control and insulin in the absence, as well as in the presence, of cyclic AMP. To determine the specificity of the protein kinase inhibition, fraction 2d was tested on three different cyclic AMP–independent protein kinases, including phosphorylase b kinase. No difference between control and insulin was observed (Table 2), indicating that the material had specificity for the cyclic AMP–dependent protein kinase. Samples of all fractions separated by Sephadex G-25 column chromatography were lyophilized for activity on mitochondrial pyruvate dehydrogenase (*11*).

Because of its ability to mimic the action of insulin on cyclic AMP–dependent protein kinase, phosphoprotein phosphatase, and pyruvate dehydrogenase, we suggest that this peptide or peptidelike substance may constitute an insulin mediator. This material may be derived in some way from insulin or from the cell membrane, although we cannot exclude an intracellular origin.

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4 September 1979

Derived X Chromosome in the Turtle Genus *Staurotypus*

Abstract. *C-banding, G-banding, and silver (Ag-AS) staining techniques reveal a distinctive sex chromosome system in the turtle* Staurotypus salvinii. *Unlike previously described systems in most other vertebrate groups in which the Y or W is derived and the homogametic sex represents the primitive condition, the reverse is true for* S. salvinii. *The X chromosome is derived; thus the homogametic sex (female) is more derived than the heterogametic sex. The male is intermediate between the female and the ancestral condition observed in other turtle species.* Staurotypus *does not conform to the general model of sex chromosome evolution for diploid dioecious organisms.*

The only known case of heteromorphic sex chromosomes in turtles was reported by Bull *et al.* (*1*) for the genus *Staurotypus* (Kinosternidae; Staurotypinae); an XX/XY sex chromosome system in which the male is the heterogametic sex. Bull *et al.* (*1*) examined three male and two female *S. salvinii* and two male and one female *S. triporcatus*. In addition, we have examined one male and three female *S. salvinii*. The X is subtelocentric, with a secondary constriction on the long arm near the centromere, and the Y is acrocentric (Fig. 1). Evidence from meiotic pairing indicates these elements are homologous along most of their length except for a short, unpaired terminal segment, probably corresponding to the secondary constriction and short arm in mitotic preparations [figure 2, c and d, in Bull *et al.* (*1*)]. We now report our studies of this sex chromosome system with C-banding, G-banding, and silver (Ag-AS) staining techniques.

Metaphase chromosomes were harvested from fibroblast cell cultures initiated from heart biopsies as described for turtles (*2, 3*). G-bands were induced by the trypsin treatment (*4*), constitutive heterochromatin was stained by Sumner's procedure (*5*), and the ammoniacal silver (Ag-AS) technique (*6*) was used to locate the nucleolar organizer regions (NOR's). The last three pairs of chromosomes in the group B complement do not band as distinctly as the first two pairs (Fig. 1) (*2, 3*); but the long arm of the sex chromosome pair in *S. salvinii* appears to be homologous to the third group B pair in *Chinemys reevesi*. The G-band negative short arms are clearly visible on the X chromosomes (Fig. 1, B and C), while the secondary constriction on the long arm is best seen in a standard karyotype [figure 1 in (*1*)] or C-band preparation (Fig. 1D). The Y chromosome is acrocentric and homologous to an acrocentric pair in *Chinemys reevesi* (Fig. 1A) and several other turtle genera (*2, 3, 7*). Our C-band preparations of several *Staurotypus* cell cultures all showed that heterochromatin is restricted to the centromeric regions except for the X chromosome, in which the short arm, the centromeric region, and the secondary constriction region of the long arm are all heterochromatic (Fig. 1D). The Ag-AS staining shows that these secondary constrictions on the X chromosomes in *S. salvinii* contain the NOR's, the locations of genes coding for 18*S* and 28*S* ribosomal RNA (Fig. 2).

An interesting feature of the sex

chromosome system of *S. salvinii* is that the X is the evolutionarily derived chromosome and the Y has remained unchanged. The X differs from the Y and from its presumed homologs in closely related and even distantly related species of turtles (including other kinosternids, testudinids, emydids, and cheloniids) by the translocation of the NOR and the addition of a heterochromatic short arm onto the X. The only other turtles known to have a modified third group B pair are *Chelydra serpentina*, which has the NOR associated with a secondary constriction on the short arm, and *Rhinoclemmys* and *Siebenrockiella crassicollis*, both of which have small heterochromatic short arms but no secondary constriction (*2*). The NOR's of other turtles examined are located on other chromosomes.

Since *Chelydra serpentina* also possesses heterochromatic short arms on its third group B chromosomes (*2*), it could represent the primitive condition for that pair. The Y chromosome in male *S. salvinii* could then be interpreted as being derived through the loss of this heterochromatic segment and secondary constriction. An independent event, the relocation of the secondary constriction from the heterochromatic short arm of *C. serpentina* to the euchromatic long arm in *S. salvinii*, would be required to derive the X chromosome of the latter species if *C. serpentina* is considered to represent the primitive condition. This does not seem to be a feasible alternative, however, because of the widespread occurrence of the acrocentric group B pair three in closely related genera of the family to which *Staurotypus* is now placed [*Sternotherus* and *Kinosternon* (*3*), the testudinid genus *Geochelone* (*2*), and at least six genera of the family Emydidae (*2*)] (Fig. 1A). Considering this widespread occurrence, the simplest assumption is that the acrocentric is the primitive condition and the X a uniquely derived element only in *S. salvinii* (through the addition of the secondary constriction and heterochromatic short arm). The condition in *C. serpentina* represents an independently derived condition.

Few other chromosome banding data are available on the sex chromosomes of other vertebrate groups with which we can compare our results, but Pathak and Stock (*8*) reported on C- and G-band analyses of 60 species of several groups of mammals. Their data showed two major G-positive regions on the X chromosome—band A (really consisting of two subbands) on the proximal portion of the long arm and band B in the middle of the short arm. These bands were conserved in all species studied, regardless of the gross morphology of the X chromosome. The X and Y chromosomes may both be modified by an inversion, autosomal translocation, or, more commonly, the addition of heterochromatic material. C-band data show that heterochromatin is usually added as an entire arm (*8*). Thus, both elements in mammalian sex chromosome systems may be derived from a more general condition.

Several types of sex chromosome systems have been described in nonmammalian vertebrates. In those possessing a ZZ/ZW chromosome system in which the female is heterogametic (birds, advanced snakes, lizards of the genera *Lacerta*, *Phyllodactylus*, and *Varanus*, and the fish *Gambusia affinis*), the W chromosome is usually interpreted as being derived (*9*). A parallel situation occurs in XX/XY systems (males heterogametic, Y chromosome derived) known in the lizard genera *Anolis*, *Cnemidophorus*, *Sceloporus*, *Scincella*, and *Uta*, and in the fish genus *Fundulus* (*10–12*). The Y is also interpreted as being derived in the $X_1X_2Y/X_1X_1X_2X_2$ systems known in the lizard genera *Anolis*, *Scelo-*

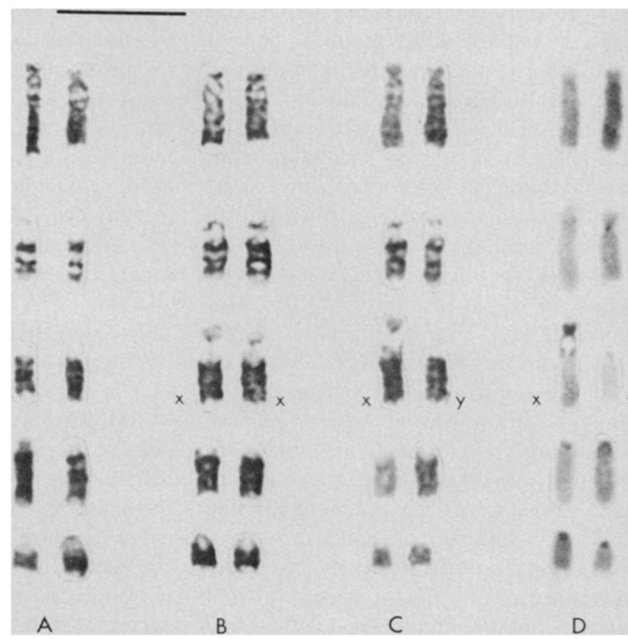

Fig. 1. G-band (A to C) and C-band (D) for (A) *Chinemys reevesi* female (Emydidae), TCWC-56736; (B) *Staurotypus salvinii* female, TCWC-55100; (C) *Staurotypus salvinii* male, TCWC-56737; and (D) *Staurotypus salvinii* male, TCWC-56737 (Kinosternidae). Bar represents 10 μm.

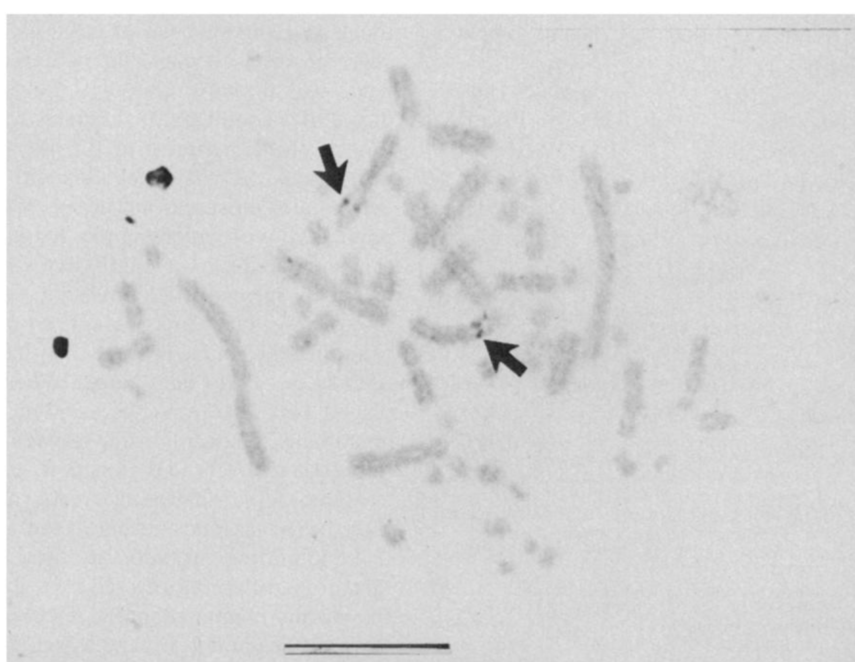

Fig. 2. Silver (Ag-AS) stained chromosomes of *Staurotypus salvinii* female (TCWC-56442) showing nucleolar organizer regions (NOR's) on secondary constrictions of the X chromosomes. Bar represents 10 μm.

porus, *Scincella*, *Polychrus*, and *Lialis*, and the fish genus *Cyprinodon* (*11–13*), while the W is derived in the $Z_1Z_1Z_2Z_2/Z_1Z_2W$ system described in the elapid snake *Bungarus caeruleus* (*14*). The common feature to all of these sex chromosome systems is that the Y or W chromosome is derived, while the homogametic sex presumably represents the ancestral condition.

The distinctive feature in *Staurotypus*, illustrated here in *S. salvinii* and presumably true also for *S. triporcatus*, is that the heterogametic sex is not the most derived. It represents an intermediate condition between the primitive condition, in which the undifferentiated ancestral pair probably resembled the acrocentric male Y and the third group B pair of most other turtles, and the most derived condition in the female having two X chromosomes derived from the autosomal translocation of the secondary constriction and a heterochromatic short arm. Thus, this system does not conform to the general model of sex chromosome evolution for diploid dioecious organisms, in which the Y (or W) becomes heterochromatic and degenerate (*15*). The Y chromosome is considered to be primitive relative to the X because it is identical in appearance to a pair of homomorphic chromosomes that are widespread in many other turtle genera and families (*2, 3, 7*). Until more banding data are available for other groups of lower vertebrates, however, we may have no way of knowing precisely what rearrangements have been involved and whether or not the Y (or W) is the only element that has been altered during the evolution of other sex chromosome systems.

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Flatworm Control of Mosquito Larvae in Rice Fields

Abstract. *We describe some flatworms (some in the genus* Mesostoma*) that kill mosquito larvae and may account for the variability in the population densities of* Culex tarsalis *and* Anopheles freeborni *in rice fields. When mosquito larvae brush against these worms, the larvae immediately become paralyzed and die. When* C. tarsalis *larvae are placed inside floating cages that exclude flatworms (50-micrometer mesh), there is a fourfold increase in their survival. Rice fields that have abundant mosquito populations lack flatworms. Most such fields have only recently been turned over to rice production, suggesting that the flatworms have difficulty dispersing to new fields but, once established, are able to overwinter and control mosquitoes for the subsequent years of rice production.*

The densities of larvae of *Culex tarsalis* and *Anopheles freeborni* from one rice field to another in the Sacramento Valley (*1*) may differ by two or three orders of magnitude (*2*). This pattern of distribution is slightly bimodal. Most of the fields have few or no mosquitoes, but a few fields virtually teem with larvae (*3*). Early investigators thought that toxins produced by blue-green algae might account for these differences, but results from laboratory and field studies have been ambiguous (*4*). Previously, we found that the survival of larval *C. tarsalis* in screened cages in various rice fields was directly related to the abundance of mosquitoes in the fields at the time of the experiment (*3, 5*). We have now discovered that rice fields with low densities of mosquito larvae are heavily populated with microscopic flatworms (many under 1 mm in length) that kill the larvae by means of a slimy toxic secretion. These flatworms are able to penetrate the 200-μm mesh screening that we used in our early field cages. When we placed two flatworms in a 300-ml cup containing 20 second-instar larvae of *C. tarsalis* and observed their activity under a stereoscopic microscope, we noted that the larvae became paralyzed (rigid and motionless) as soon as they contacted one of the worms (Fig. 1). Later, the worms might recontact a paralyzed larva and feed on it, but the worms killed many more larvae than they ate. In fact, all the larvae were killed within 2 hours.

Extensive field sampling and laboratory identification revealed that as many as eight types (species?) of flatworms may be present in the rice fields. The predominant flatworm in these fields was found to be a *Mesostoma* species, a turbellarian in the order Rhabdocoela (*6*). Certain species in this order possess epidermal rhabdoids (rhabdites) that are slightly curved rods shorter than the height of the epidermis (*7*). These rods are secreted by gland cells and are discharged to the surface as a toxic mucous secretion that is repellent to predators (*8*). Although a few literature citations document the toxicity of some flatworm secretions, the extreme potency of the rice field species appears unprecedented (*7–9*).

We identified 14 rice fields in Sutter, Yuba, and Sacramento counties that differed in aquatic fauna, flora, and physical attributes, and placed four floating cages in each field (*10*). We stocked the cages with 25 third-instar larvae of *C. tarsalis* bred in the laboratory, and capped each cage with a tight organdy top. In two of the cages we placed 0.2 ml of Tetramin (a commercial fish food used successfully as larval food in the laboratory) every alternate day; the larvae in the other two cages in each field received no supplemental food and served as controls. Each day the dead and live larvae, pupae, and adults were counted, and the dead ones were removed.

After 1 week an average (over all cages in all fields) of 51 percent of the larval mosquitoes had died in the Tetra-

