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Sex Identification in Young Kemp's Ridley Sea Turtles

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Temperature-dependent sex determination, homomorphic sex chromosomes and the absence of sexual dimorphism in young sea turtles limit sexing of individuals to a sacrificial method involving histological examination of gonads. As a result it is not possible to determine the relationship between sex development and environment in the critically endangered Kemp's ridley sea turtle (*Lepidochelys kempi*). Furthermore, managers cannot determine sex ratios in wild populations of Kemp's ridleys containing high percentages of juveniles, headstart programs have no knowledge of sex ratio of turtles hatched (except those that die) or released, and captive propagation operations must retain individuals for years before selecting for future brood-stock.

Previous non-sacrificial attempts to sex immature sea turtles have been based on meristics (A. Landry, personal communication), H-Y antigen (Wellins, 1987), laparoscopy and prepubertal levels of sex steroids. The most useful technique is radioimmunoassay of steroids because testosterone levels are higher in males than in females (Owens et al., 1978). However, sex is identified correctly only 90% of the time and the animals must be at least two years old (D. Owens, personal communication, 1987).

In an effort to provide a non-sacrificial means of identifying genotypic sex of hatchling Kemp's ridleys, we are pursuing two avenues of investigation, one molecular and the other immunologic. The first involves determining whether specific deoxyribonucleic acid (DNA) sequences of the sex chromosomes can be detected. A DNA probe (BKm - a laboratory detectable sequence of sex specific minor DNA) was obtained from the W chromosome of a venomous Indian snake (*Bungarus fasciatus*). This probe detects homologous sequences on the W chromosome of other snakes (even in species that carry homomorphic Z and W chromosomes), on the W chromosome of birds and on the Y chromosome of mammals (Singh et al., 1976, 1980). We are attempting to detect such sequences in Kemp's ridley.

Twelve animals of known sex were used. Eight (5 females, 3 males) were 9-yr olds maintained from hatchling stage at an oceanarium. They were sexed by laparoscopy. Four (3 females, 1 male) were head-started yearlings that had to be euthanized because of gross carapace and plastron malformations. These were sexed histologically. A small sample of blood (0.5 ml or less) was obtained from each animal and DNA was extracted from the cells. The DNA was then digested with a restriction endonuclease and the fragments displayed by size using agarose gel electrophoresis. The DNA was transferred and fixed to membrane filters and the filters exposed to biotin-labelled BKm probe DNA. Homologous sequences are manifested as "bands" on the membrane filter. We have identified several bands that are sex-specific or exhibit a dose effect. Because the turtles are not inbred, more individuals will have to be sampled in order to rule out autosomal polymorphism.

The second avenue of study is to use an antibody that will signal the presence of H-Y antigen on Kemp's ridley blood cells. H-Y antigen is a cell surface protein expressed sex-specifically in all vertebrates examined (Wachtel, 1983). We have produced several monoclonal H-Y antibodies and currently are devising a technique to determine whether H-Y is present on blood cells of Kemp's ridley, and whether it is expressed sex-specifically. An Elisa will allow either direct detection of H-Y on the cells, or expression of H-Y will be inferred by absorbing H-Y antisera with turtle blood cells and assaying those treated antisera for residual anti-H-Y activity using a known H-Y source as an antigen. The immunologic technique is much more cost-effective than the molecular method and the results would be available in hours rather than in days.

Although our results are preliminary, we are encouraged that a non-sacrificial method for determining the genotype sex of young Kemp's ridleys is forthcoming. Development of this non-sacrificial method will make it possible to determine with accuracy the sex ratio of hatchlings produced in conservation programs, facilitate studies on the effects of environment on sex development and increase the efficiency of broodstock selection on turtle farms. These techniques would prove most useful if they can be applied in other marine turtle species in the hatchling and juvenile stages. Finally, these studies will allow us to conduct population studies to determine the size of the gene pool and species relationships.

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