Regeneration of gill lamellae of the Indian horseshoe crab, *Tachypleus gigas* (Müller)

In marine organisms, regeneration of lost tissue is considered to be a natural phenomenon[1,2]. Most of the work reported on regeneration of lost parts are related to predation. In natural environment it has been observed that the regeneration process is not only species-specific but is also dependent on seasons. There have been few records showing high regeneration rate in warmer months[3]. A clear picture of the regeneration process has been demonstrated by conducting experiments on brittle stars in the laboratory[3,4]. The regeneration process in the laboratory has been found to be temperature-dependent and is completed within 100 to 200 days[5]. Attempts have also been made to study the regeneration process at the molecular level[6,7]. However, due to lack of knowledge of molecular and genetic manipulations of the commonly studied vertebrate models, not much success has been achieved so far. Sanchez and Newmark[6] made an attempt to study the introduction of double-stranded RNA that abolished the gene function in planarians, which is a classical example of the regeneration process. An extensive study has also been carried out on the genes and molecules involved in the regeneration process in planarians[7].

Two species of horseshoe crab, namely *Tachypleus gigas* (Müller) and *Carcinoscorpius rotundicauda* (Latreille) are abundantly found along the northeast coast of India[8]. The carapace of the horseshoe crab consists of two parts, viz. prosoma and opisthosoma. The opisthosoma is a hexagonal, broad plate connected to the carapace by a flexible joint, allowing it to move up and down. There are broad, flat, thin and double membrane structures attached on the underside of the opisthosoma. As these structures look like pages of a book, they are called book gills or gill lamellae. More than a thousand gill lamellae are present on either side of the opisthosoma. A well-developed circulating system with blood vessels in the gill books helps the animal in oxygen exchange. As long as the gills are moist, the horseshoe crab can get oxygen from the air while they are on the beaches for breeding. The gill books can be opened and moved easily in such a manner that they help young horseshoe crabs to swim upside down. These gill lamellae are an important source of amoebocytes in horseshoe crabs[9].

A clotting enzyme popularly known as amoebocyte lysate has been discovered in the amoebocytes of the horseshoe crab, which clots and forms a firm opaque gel, immediately on exposure to minute amount of endotoxin or bacterial pyrogens[10]. The rate of gelation is directly related to the concentration of endotoxins and protein content of the amoebocyte lysate. Amoebocyte lysate is now used as a fast, effective way of testing drugs to make sure they are free of harmful bacteria, before they are administered to patients.

Though the commercial production and biomedical application of lysate test has become more standardized in recent years, significant variation has been observed in the quality and sensitivity of amoebocyte lysate if it is produced from wild population[11]. This is basically on account of compositional variability of the haemolymph during monsoon season. Considering the seasonal and batch-to-batch variability in the sensitivity of amoebocyte lysate and threat to the horseshoe crab population, an attempt has been made in India to cultivate amoebocyte-producing tissue *in vitro*[12]. This investigation was undertaken to eliminate the collection of amoebocytes from the wild stock. In our study, about 200 gill lamellae were removed for *in vitro* cultivation for mass production of amoebocytes. During our experiment, we had observed that within 3–4 months, the lost gill lamellae were completely regenerated. Considering this as an important phenomenon, a detailed study was carried out to examine the regeneration rate of the gill lamellae of the Indian horseshoe crab, as information on the regeneration of lost parts has not been reported so far.

For the present study, live adult horseshoe crabs were collected from the breeding beach in Orissa (lat 21°27′N; long 87°04′E). The specimens were transported to the laboratory in Goa in specially designed containers and maintained in fiberglass tanks (capacity 2000 l). The fiberglass tanks were provided with a sand bed (grain size: 65–125 μm) and continuous supply of re-circulating filtered sea water (0.5 μm) of 30‰ salinity. In our study, about 200 gill lamellae were removed from ten adult horseshoe crabs for *in vitro* cultivation of tissue to harvest amoebocytes. The dissected part of the animal was washed with antibiotic (5% betadine solution) and 70% alcohol. Specimens with lost gill lamellae were tagged and kept separately in the laboratory in fiberglass tanks. Temperature of the water was maintained at about 27°C by thermostatically regulated titanium heaters throughout the period of experiment. The animals were observed weekly for a period of 90–125 days for regeneration of gill lamellae.

In our experiment, about a third of the lost gill lamellae was observed to be regenerated on the 50th day and half on the 75th day (Figure 1). The total regeneration of the gill lamellae was observed on day 125 (Figure 2 *a* and *b*).

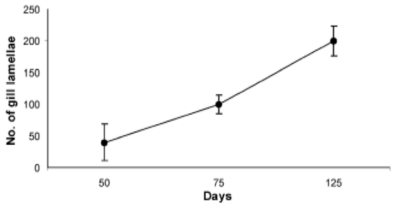

Figure 1. Regeneration of gill lamellae.

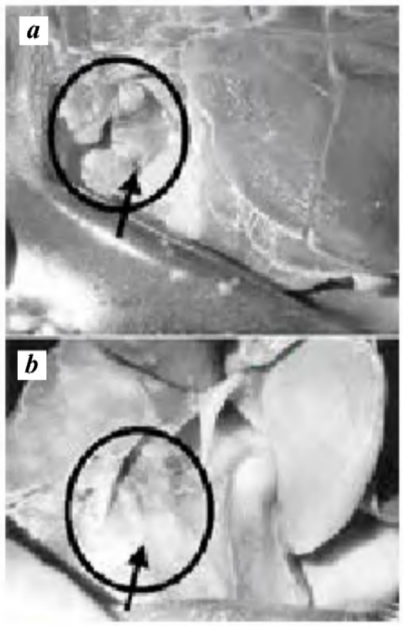

Figure 2. ***a***, Arrow shows incised gill lamellae (in circle). ***b***, Arrow shows regenerated gill lamellae (in circle).

Regeneration is a process that requires more energy compared to that required for growth of the animal[13]. Marine animals are capable of differentially allocating nutrients to different functions of body such as maintenance, metabolism, somatic growth, wound repair and reproductive tissue growth. The allocation is a dynamic process depending on the physiological and reproductive states of the organisms[14,15].

It has been a well-known fact that the crustaceans are able to re-grow their limbs and regenerate muscle fibres. The discovery of the factors responsible for regeneration may provide vital information to determine how to replicate such a process with human tissues. The implications of this for human health are enormous because crustacean muscles seem remarkably similar to humans. Protein structure in human is almost the same and the crustacean molecular switches work the same way as human muscles do.

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Occurrence of *Ginkgo* Linn. in Early Cretaceous deposits of South Rewa Basin, Madhya Pradesh

Ginkgo biloba Linn. (maiden-hair tree), the only extant species of Ginkgoales shows several ancestors in fossil records. It is the sole living member among the race of dominant plants that probably existed on the earth longer than any other tree. It has a long fossil history ranging from the Late Permian (270–255 million years) to modern time and achieved its maximum diversity during the Jurassic (240–255 m.a.) period[1,2]. They have mostly been reported from the northern hemisphere, but also occur in the Gondwana deposits[3]. In India, the earliest record goes back to the Permian of the Rajmahal Basin[4]. The Triassic beds of South Rewa Basin also yield *Ginkgo*-like leaves[2,5,6], but definite Ginkgoales were recorded from Jurassic beds of Rajmahal Basin[7]. Many forms of leaves and ovules of *Ginkgo* from the Early Cretaceous deposits are virtually unchanged till modern time.

The order Ginkgoales in the Mesozoic deposits is represented by leaf remains and ovules attributed to *Ginkgo* and *Ginkgoites*. Although quite meagre in fossil forms, their records indicate that they were morphologically diversified before the Early Cretaceous. Therefore, we can say that the Mesozoic witnessed the zenith of Ginkgoales along with other plant groups like Cycadales, Bennettitales and Coniferales. The other plant groups declined during Middle Cretaceous and most of them became extinct before the end of the Cretaceous[8], but Ginkgoales along with Coniferales and Cycadales survived in later periods. The Early Cretaceous remains of Ginkgoales represent a few species which show much similarity as the fossil and modern taxa of other plant groups like Coniferales and Cycadales. The appearance of Ginkgoales in India during Early Cretaceous is significant. Therefore, the present study is aimed to trace its lineage from the earliest record of fossils from the Permian to Early Cretaceous.

In context to the Indian fossil wealth, the appearance of Ginkgoalean leaves occurred during Late Permian[4]: *Rhipidopsis densinervis*, *R. gondwanensis*, *Ginkgoites veekaysinghii*, *G. huraensis*, *Saportaea reniformoides* and *Psygmophyllum kidstonii*. The Triassic genera reported from Goira (Shahdol District, Madhya Pradesh (MP) is *Ginkgoites goiraensis*[6] and *Ginkgo*-like leaves from South Rewa Gondwana Basin[2]. The Jurassic forms of *Ginkgo* are reported from Rajmahal Hills, i.e. *Ginkgo rajmahlensis*[9–11]. Later, records were made from different Early Cretaceous beds: *Ginkgoites lobata* from Satpura Basin[12,13], *G. crassipes* from Sriperambudur beds[13] and *G. cressipes*[14] and *G. feistmantalii*[15] from Bansa, South Rewa Basin. *G. feistamantli* was also reported from Ragha-