when the nature of psychoneuroimmune interaction in fish is delineated when we get more information on the fish pheromones and their mode of action on immune system.

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# Lamellar angiography, a novel method for gill respiratory area measurement: SEM of gill corrosion vascular replica

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**Measurement of lamellar dimensions of the swamp catfish, *Chaca chaca* has been made using corrosion vascular replica. Advantage of corrosion vascular cast for sampling lamellae for their measurements is discussed in the light of data obtained from Bouin's fixed materials.**

FISH gill lammellae are the sites for gaseous exchange and their measurement is subjected to various methodological errors because of the heterogeneity in the dimensions of a large number of lamellae[1]. It is impossibly difficult to measure the area of all the gill lamellae immediately after their removal from the anaesthetized fish to obtain the absolute lamellar values. Formalin- and Bouin's-fixed gills are commonly used for surface area measurements[2]. In recent years, Bouin's-fixed gills have been used for lamellar measurements because it fixes and stains as well. Shrinkage due to fixation is one of the greatest sources of error. The other source of error is the inclusion of nonrespiratory pillar cell system in the lamellar measurements of fresh and fixed gills. To get rid of these sources of errors, an attempt has been made to sample lamellae of *Chaca chaca* injected with methyl methacrylate resin for estimating only the lamellar blood channels, which are the actual functional sites for gaseous exchange.

*Chaca chaca* belongs to the family Chacidae of the order Siluriformes and is well adapted to hypoxic swamp (2 mg $O_2$/l) infested with macrovegetation and decaying organic matter. Live specimens of *C. chaca* were collected from the swamp near Purnia (Bihar), and maintained in plastic tanks (40 l) in the laboratory. Live specimens ($n = 3$) of *C. chaca* were anaesthetized by MS 222, ventral aorta was cannulated and phosphate-buffered Ringer's solution with 100 USP/ml heparin, was infused at physiological pressure of 30 mm Hg to fill the lumen of the cardiovascular system by replacing blood. Methyl methacrylate (Mercox) was mixed with catalyst and infused also at 30 mm Hg (ref. 3). At the onset of polymerization, the ventral aorta was clamped and the fish was placed in 60°C tapwater for 2 h to ensure complete polymerization. The vascular replica was obtained after treating the carcass with 20% NaOH, water and 3% $HNO_3$. Vascular replicas of gill filaments of the sampled fishes were mounted on an SEM stub with silver paste, gold sputtered and examined with P-SEM 500 scanning electron microscope.

Scanning electron micrographs (SEMs) of the sampled lamellae ($n = 15$) from base, middle and top of the filamentar vascular replica were projected on $mm^2$ rectilinear grid to measure their dimensions. The data were compared with those from Bouin's-fixed gills of *C. chaca* ($62 \pm 2$ g) body weight. Paired $t$ test was employed to test the level of significance between the mean lamellar area values of the lamellae sampled from vascular replica and those from Bouin's-fixed gills.

Respiratory and nutritive vascular systems are discernible in the angioarchitecture of the gill filament (Figure 1). The marginal channel and the central vascular network of a lamella constitute its respiratory part, whereas the network of blood channels of the vascular replica of the gill filament is its nutritive part. The former takes care of the gaseous exchange between the $O_2$ present in the ambient water and the blood that circulates through the marginal and the central network of the lamellar channels and the latter provides nourishment and $O_2$ to the underlying tissues of the gill filaments. The afferent and efferent sides of the lamellar vascular replica are differentiated by narrow and wider profiles respectively (Figure 1 *b*). Flow of blood from afferent to efferent ends through the vascular network and the up-inclined marginal channel results in lower velocity of blood flow, which allows the haemoglobin greater $O_2$ loading from the counter-current flow of ventilating water from leading to trailing edges of the inter-lamellar spaces. The total vascular area of an average lamellar replica is about $0.11328 \pm 0.01011$ $mm^2$. This value is significantly ($P < 0.005$) higher (63.298%) than the

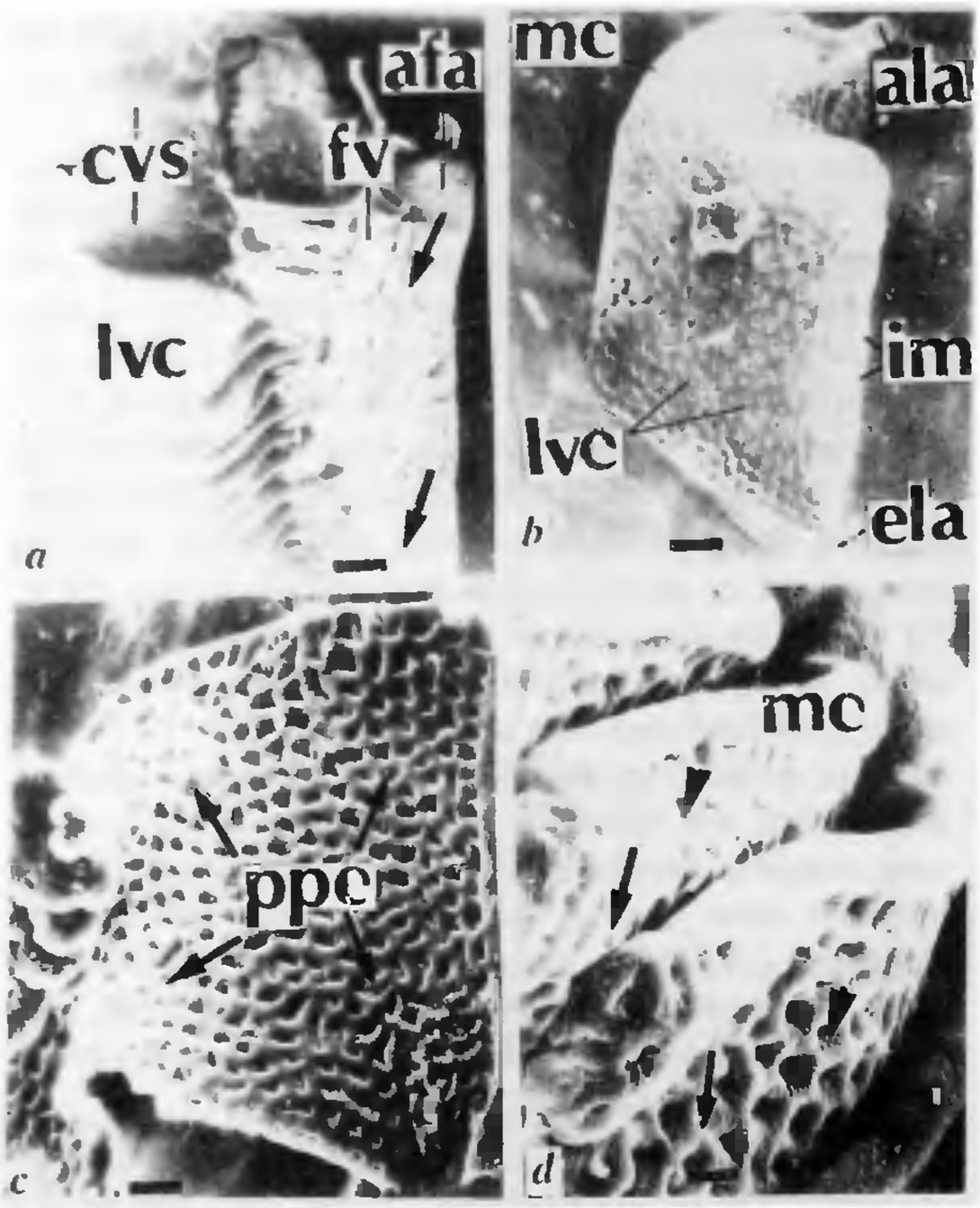


**Figure 1.** *a*, SEM of a part of gill filament vascular cast showing afferent filamentar artery (afa), central venous sinus (cvs), filamentar vein (fv) and lamellar vascular cast (lvc), bar = 90 µm. *b*, SEM of a lamellar vascular cast (lvc) showing afferent lamellar arteriole (ala), efferent lamellar arteriole (ela), inner margin (im) and marginal channel (mc) of a lamella, bar = 42 µm. *c*, SEM of a part of lamellar vascular cast showing position of pillar cells (ppc), bar = 21 µm. *d*, SEM of parts of three lamellar vascular cast showing marginal channels (mc), their ramification (arrows) and the position of pillar cells (arrowheads), bar = 12 µm.

value ($0.06937 \pm 0.01633$ mm$^2$) obtained from Bouin's-fixed lamellae of about $62 \pm 2$ g *Chaca*[4]. Out of the total angio-lamellar replica area ($0.11328 \pm 0.01011$ mm$^2$), about 39% ($0.04458 \pm 0.01011$ mm$^2$) is occupied by pillar cell system (6721 mm$^2$) and the remainder (61%) ($0.06937 \pm 0.01633$ mm$^2$) by blood channels. Out of the total blood channel area ($0.06937 \pm 0.01633$), about 30.6% is occupied by the marginal channel and 69.4% by a network of central lamellar blood channels. 30.6% of the blood flow through the marginal channel is of great respiratory importance because of its smaller water–blood diffusion distance and greater diffusing capacity in comparison to the other parts of the gill lamellae[5]. The 39% pillar cell system does not participate in gaseous exchange and therefore should not be considered for the measurement of total respiratory area of a lamella. Measurement of total respiratory lamellar area of fresh- and fixed-gills includes the pillar cell system also and overestimates the respiratory surface. From the above findings it may be suggested that measurements of gill area should be made with the help of bilateral surface area of the lamellae sampled from corrosion vascular replica of the gill filaments.

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# Regeneration of Indian cotton variety MCU-5 through somatic embryogenesis

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***In vitro* regeneration by somatic embryogenesis is highly varietal specific in cotton (*Gossypium hirsutum*). Highest frequency of regeneration has been reported in Coker varieties. However, even within Coker varieties, there is seed to seed variation for regeneration[1,2]. There is no report of *in vitro* regeneration of major Indian cultivars through the somatic embryogenesis pathway. We report here identification of genotypes within variety MCU-5 that regenerate profusely by somatic embryogenesis. This variety is extensively grown in the southern cotton zone of India. Regeneration by somatic embryogenesis has been achieved by a modification of the earlier *in vitro* culture protocols of Trolinder and Goodin[3] and Firoozabady and DeBoer[4]. Complete plants could be regenerated through somatic embryogenesis from hypocotyl explants in 6–7 months.**

SOMATIC embryogenesis was first observed by Price and Smith[5] in *Gossypium koltzchianum* but no plantlet regeneration was reported. Shoemaker *et al.*[6] described somatic embryogenesis and plant regeneration in *G. hirsutum* cvs Coker 201 and 315. Since then, significant progress has been reported in the regeneration of Coker

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