Discrimination of Genetic Sex in Embryos of d-*rR* Strain of the Medaka (*Oryzias latipes*)

The discrimination of genetic male and female in animal embryos is not easy. No example of the discrimination in fish embryos has ever been reported. The sex chromosome constitution normal to the medaka (*Oryzias latipes*), an oviparous toothcarp, is *XX* for female and *XY* for male and the *R* or *r* gene responsible for orange-red colour (carotenoid) may be borne by either the *X* or *Y* chromosome[1]. The d-*rR* strain of the fish, where females are white (X^rX^r) and males orange-red (X^rY^R) has been established by YAMAMOTO[2]. Thus, the sex-genotype can be discriminated by the body colour of fish in this breed. The disturbance of the *Y*-linked inheritance by both crossing over between the *r* and *R* and genic imbalance between autosomal and allosomal sex-genes is extremely rare; less than 1%[3]. Thus the determination of sex-genotype by body colour in the d-*rR* strain is possible with a high reliability.

The white medakas (*rr*) have little carotenoid in their xanthophores while the orange-red medakas (*RR* or *Rr*) have xanthophores with carotenoid. However, *r* and *R* fish can only be distinguished after hatching in normal breeding. Using the d-*rR* stock, a method of discrimination of the sex-genotype in embryos of this fish is devised. If the egg of the orange-red medaka has sufficient carotenoid, the developed embryo has diffused colour (dorsal colour) of carotenoid nature[4] and unknown light yellow pigment on the head and dorsal part of the trunk but the embryo of the white variety (*rr*) has only light yellow dorsal colour. Consequently, it is possible to discriminate sex-genotype of embryos of the d-*rR* medaka by the dorsal colour.

All eggs used in the present experiment were injected red carotenoid of red pepper (*Capsicum annuum*) into the yolk shortly after artificial fertilization[5] by the method of TAKEUCHI[6]. In order to obtain dorsal colour of embryos by carotenoid, injected pigment in the yolk must be absorbed before hatching. Carotenoid-Tween 80 complex is found to be an excellent material as injection solution for the purpose. The egg membrane was removed to observe the dorsal colour and the dorsal colour on the head is primarily used for the discrimination. Embryonic stages are expressed by the stage number of MATSUI[7]. The dorsal colour caused by red carotenoid appears at stage 29 in the orange-red variety when the tail of an embryo reaches the eye and the dorsal colour reaches maximum visibility at the stage 31 (Figure 1). The dorsal colour fades out after hatching as aggregated xanthophores become visible. On the other hand, no reddish dorsal colour appears until stage 32 in the white variety and a little reddish dorsal colour is visible at stage 33 shortly before hatching. Consequently, the discrimination of the orange-red medaka from the white is better performed at the stage 31. In other words, a genetic male (X^rY^R) medaka can be discriminated from a genetic female (X^rX^r) at this stage.

The discrimination was performed in the embryos of d-*rR* strain and 27 expected males and 25 expected females were obtained. The discrimination can be confirmed with the colouration of xanthophores by using the following fact. Although an embryo before hatching has usually no aggregated xanthophores, they appear in an isolated piece (Figure 2) of an embryo of the orange-red breed, older than stage 26 (black pigment appears in the eye at this stage), cultured several days at 25 °C in the 1/7.5 *M* balanced salt solution formulated by YAMAMOTO[5] on the basis of osmotic pressure[8]. Many deeply coloured aggregated xanthophores are observed, if a piece of embryo older than stage 30 is cultured. However, if a piece of an embryo of the white variety at various stages is cultured, no coloured aggregated xanthophores are observed except with an embryo at stage 33. A piece of an embryo of white variety at stage 33 has slightly coloured xanthophores after a few days' culture.

The 27 expected orange-red embryos (male) and 25 expected white embryos (female) mentioned above were cultured in the balanced salt solution at 25 °C and the colouration of xanthophores was examined after several days. Of 27 expected males, 26 had deeply or moderately coloured xanthophores and only 1 had no coloured xanthophores, indicating only 4% error. Of 25 expected

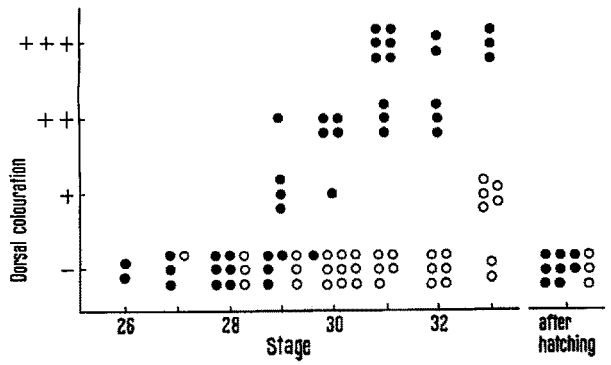

Fig. 1. Reddish colouration in the dorsal part of an embryo. +++, ++, + and – indicate deep, moderate, slight and no colouration respectively. Stages are the stage numbers of MATSUI[7]. •, orange-red variety; o, white variety. All eggs are injected red carotenoid shortly after fertilization. A point corresponds to an embryo.

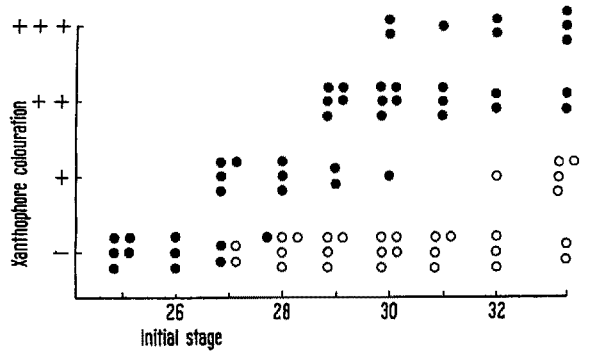

Fig. 2. The colouration of xanthophores of a cultured embryo. +++, ++, + and – indicate deeply, moderately, slightly and no coloured xanthophores respectively. Stages at the operation for culture are on the abscissa. •, orange-red variety; o, white variety. All eggs are injected red carotenoid shortly after fertilization. A point corresponds to an embryo.

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females, 19 had no coloured, 4 had slightly coloured and 2 had moderately coloured xanthophores, indicating 8% error. In conclusion, the error in the discrimination of genetic sex in the d-*rR* medaka embryos by the present method is less than 10%[9].

Zusammenfassung. Die Körperfarbe des männlichen d-*rR* Medaka ist wegen des in Xanthophor enthaltenen Carotinoid-Pigments orange, die der weiblichen wegen seiner Abwesenheit uni. Die Färbung erfolgt im allgemeinen nach der Ausbrütung. Wird das rote Carotinoid des Paprikas unmittelbar nach der Befruchtung injiziert, so wird die dorsale Körperoberfläche des männlichen Embryos durch das Pigment gefärbt, was die Feststellung des erblichen Geschlechts schon vor der Ausbrütung erlaubt. Beurteilungsfehler konnten bis auf weniger als 10% herabgedrückt werden.

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Contractility Cycle of an Isolated Gastropod Ventricle[1]

Molluscan and vertebrate cardiac muscle differ in at least one of the properties that are said to make discussion of mechanical properties of the latter difficult, if couched in the terms developed for skeletal muscle. Those properties of vertebrate cardiac muscle are: (1) presence of 'appreciable resting tension...at all lengths at which active tension is developed'[2]; (2) marked dependence of isometric tension on length throughout the length-tension diagram[2-4]; (3) 'time dependence of onset of active state'[5] and (4) that normal cardiac muscle cannot be tetanized and therefore cannot provide a measure of maximum contractility independent of time[3].

It has been known since the last century that molluscan cardiac muscle can be tetanized[6]. If it were established that molluscan and vertebrate myocardium were alike in property (3), molluscan cardiac muscle would offer a means of studying a contractile tissue with slow onset of 'active state' in terms meaningful with regard to the body of work on skeletal muscle, for which the onset of active state is abrupt[7].

The method of following the course of activation of contractility by quick release[8] has been applied by BLEICHERT et al.[9] to spontaneously beating rings made from the ventricle of the heart of a species of *Aplysia* (Opisthobranchia Anaspidea). Their results may be interpreted as showing that active state increased during the first third of an isometric single contraction, remained constant until shortly before peak tension, fell sharply at the peak, and shortly thereafter became zero. Their finding that at least in *Aplysia* (and presumably in other anaspid opisthobranchs) the heart muscle is extraordinarily plastic, developing the same tension over a 10 times change in length, suggests property (2) is not so marked for mollusc hearts. However, the mollusca possess great diversity in cardiac architecture and consequent strength of the ventricular wall[6]. This report deals with the time course of active state, as determined by quick stretch and quick release techniques, in the isolated perfused ventricle of a mollusc with a robust heart, *Busycon canaliculatum* (Prosobranchia).

Methods. Before each experiment the ventricle in its bath was stretched vertically between aorta and auricle to a chosen initial tension such that fibers in trabeculae oriented in that axis[10] would contract in essentially isometric fashion, although the ventricle could beat spontaneously since the contraction of circularly oriented fibers was essentially isotonic. Spontaneous beating was maintained by perfusion with aerated sea water at a head of 60 cm H_2O through a cannula in the auricle. Quick stretches and releases were given by a Levin-Wyman ergometer adapted so that tension was detected by a RCA 5734 mechano-electronic transducer mounted at the end of the moving beam of the ergometer. The plate shaft of the transducer was connected by a nylon thread to the aorta of the *Busycon* ventricle in such a way that the aorta was not occluded. Movement of the ergometer beam was detected by the use of an Ether Ltd. PD 20 displacement transducer, and output of both transducers was displayed simultaneously on a dual beam cathode ray oscilloscope. All experiments were performed during June, July, and August of 1966 at room temperature of 23–29 °C. 42 preparations were used.

Results. Figure 1 is a photographic record of a representative series of quick (5 msec) releases to zero tension. These 4 releases were chosen to represent releases at half-systole, two-thirds systole, full systole and one-third diastole from among 46 quick releases of 0.75 mm given to one ventricle at 25 °C. Redevelopment of tension is maximal following release early in the cardiac cycle (Figure 2 b) but the capacity to redevelop tension persists until quite late in diastole (Figure 2 e). If a release is given during diastolic pause, the next contraction develops the lower tension characteristic of the shorter length (Figure 2 a). Thus giving a quick release during a beat shifts the regenerated beat to the tension curve characteristic of the new length; the amount of tension redeveloped being dependent on how much of the time course of the beat is still to run. Similarly, after a quick stretch we find that the isometric contraction proceeds along its original time

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