avoided phagocytosis by normal murine macrophages. Organisms of this latter subpopulation remained in the supernatant of cultures of L929 cells or macrophages for prolonged periods of time and when inoculated into mice produced infections with parasitemias and survival times that were significantly lower and longer than those noted in mice inoculated with preparations containing trypanosomes of both subpopulations. This observation is in agreement with the report by Howells and Chiari[17] who showed that mice inoculated with trypanosomes recovered from the blood of other mice inoculated with the organisms two days before had longer prepatent parasitemias and increased survival times than controls. In addition, when trypanosomes of the 'non entering' population were added to acellular medium and cultured at 28 °C they immediately started multiplying and a slight increase in the number of organisms could be noted as early as 36 h of culture. In contrast, when a control preparation containing organisms of both populations was similarly cultured a decrease in the number of organisms was noted at 36 h of culture. The decrease in the number of organisms was not statistically significant but may have indicated death of those trypanosomes that were unable to differentiate into epimastigotes and multiply as such, possibly because they had been biologically programed to invade cells and maintain the infection in the mammalian host. So far, attempts to identify a particular subpopulation of parasites responsible for maintenance of the infection in mammalian hosts have not been successfull. The clonal approach has revealed the existence of differences among individual parasites in regard to invasiveness and pathogenicity[5]. Comparison of trypanosomes obtained from insect vectors, from bloodstream of mammalian hosts, from cell cultures or from axenic cultures have also demonstrated differences in invasiveness and in lethality[11, 17–19].

Long term maintenance of parasite strains in cultures or in laboratory mammalian hosts selects organisms with the highest ability to adapt to the culture conditions or to the experimental host but do not change the genetic make-up of individual organisms[20, 21]. Heterogeneity, however, has to be intrinsic to each organism and its expression genetically regulated since differences present in isolates kept in the laboratory for long periods of time were also demonstrated in parasites derived from a single clone.

At the moment, lectins are being used in our laboratory to examine the changes that occur on the cell membrane surface of trypanosomes following their retrieval from mammalian hosts and culturing until their final differentiation into epimastigotes.

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Asymmetric suction feeding in primitive salamanders

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Summary. During suction feeding, the primitive aquatic salamanders *Cryptobranchus alleganiensis* and *Andrias japonicus* frequently display asymmetric movements of the lower jaw and hyoid apparatus that have some similarity to working and balancing side kinematics of mammalian mastication.
Key words. Salamander; feeding; asymmetry.

Previous detailed studies of suction feeding in vertebrates have demonstrated, or assumed, that the right and left visceral arch elements of the head (jaws, hyoid and branchial arches) move symmetrically[1–6]. Suction feeding usually entails very rapid movements of the feeding apparatus[7], even in salamanders[5, 6], and is characterized by marked overlap in the activity of antagonistic muscles[1, 5, 8, 9]. We were therefore surprised to discover asymmetries in jaw and hyoid movements during rapid suction feeding in the large, primitive aquatic salamanders *Cryptobranchus alleganiensis* and *Andrias japonicus*.

Cryptobranchus alleganiensis lives in unpolluted streams and rivers of the Appalachian and Ozark regions of the United States and feeds on a variety of aquatic invertebrates and small vertebrates[10, 11]. *Andrias japonicus* inhabits montane streams of the Japanese island of Hondo and exhibits food and habitat requirements similar to those of *Cryptobranchus*[12]. We here report the nature of asymmetric movements of the feeding apparatus in these two species, the structural basis of these movements in *Cryptobranchus*, and the potential relevance of our findings to ideas concerning the evolution of vertebrate feeding mechanics.

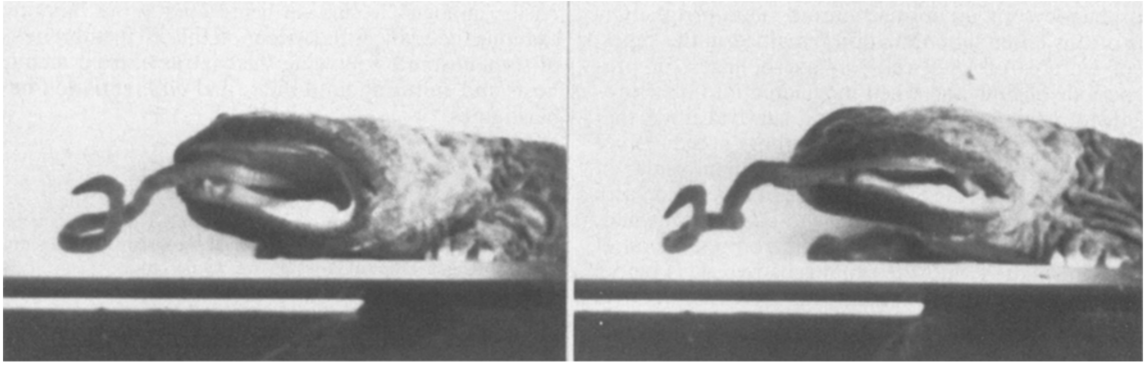

Figure 1. Anterolateral views of asymmetric jaw closure during a manipulatory cycle taken from two consecutive frames of Super-8 film shot at 24 fps.

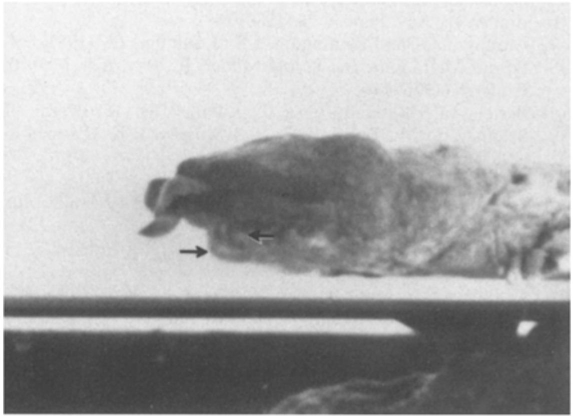

Figure 2. Lateral view of an asymmetric hyoid position achieved during a manipulatory cycle. Arrows point to positions of right and left hypohyals (see fig. 4).

Methods. Jaw and hyoid movements were analyzed from film records (strobe-synchronized Super-8 at 24 or 36 frames per second and high-speed video at 120 fps) of three *Cryptobranchus* (total lengths of 21, 51 and 56 cm, all caught at French Creek, Pennsylvania) feeding on crickets and earthworms, and two *Andrias* (total lengths 59 and 62 cm, captive reared) feeding on freshly killed small mice.

To determine the structural basis of asymmetric jaw and hyoid movements, we dissected eight *Cryptobranchus alleganiensis*. In addition, the symphyseal soft tissues of two specimens were removed, sectioned and stained for elastin[13] or collagenous connective tissue[14]. Potential movements of the different parts of the cephalic skeleton were determined by manual manipulation of seven live *Cryptobranchus* anesthetized with tricaine methanesulfonate. Photographic and radiographic records of these manipulations were compared with measurements made directly on the animal.

Results. The basic patterns of suction feeding in *Cryptobranchus* and *Andrias* were similar to those in other suction feeding salamanders[5,6]. Duration of complete gape cycles during prey capture varied from 32 to 96 ms. After capture, prey were manipulated using movements of the jaws and hyoid apparatus which were similar to movements used in capture but varied considerably in timing and extent.

During both prey capture and manipulation, the mandibles of *Cryptobranchus* sometimes flexed around the mandibular symphysis by as much as 40°. The result of this flexion was quite spectacular, the animal appearing to open or close one side of its mouth almost independently of the other side (fig. 1). This behavior was exhibited often enough (14 (42%) of 33 analyzable prey capture sequences, 170 (42%) of 410 analyzable manipulation sequences) to indicate that it is a normal function of the feeding apparatus in this species. Further, in 24% of manipulation sequences showing asymmetric jaw movements, hyoid movements were also asymmetric (fig. 2).

Andrias displayed asymmetric movements similar in frequency and timing to those of *Cryptobranchus*. However, flexion around the mandibular symphysis rarely exceeded 20°.

Asymmetric mandibular depression during prey capture was directly correlated with prey position relative to the head. In those sequences showing asymmetric movement, the mandible on the side of the head closest to the prey opened earlier and further than the opposite mandible. Asymmetric mandibular or hyoid movements were less clearly correlated with the position of prey in the jaws or buccal cavity. Marked asymmetric movements were exhibited by both larger *Cryptobranchus* during feeding on earthworms which had a mass insufficient to cause any direct mechanical effect on the hyoid or mandible. Worms were occasionally held by the teeth of the maxilla and dentary of one side while the opposite side exhibited gaping (chewing?) motions. Like the lungfish *Lepidosiren*[9], these salamanders invariably spent

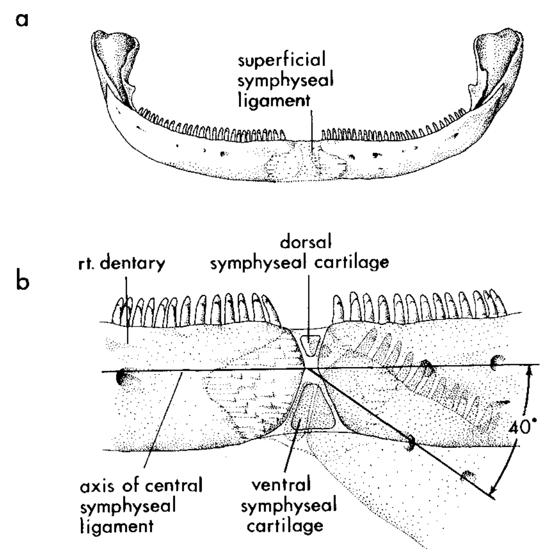

Figure 3. *a* Anterior view of the lower jaw of *C. alleganiensis*. *b* Enlargement of the symphyseal region of (a) to show positions of cartilaginous symphyseal pads and their probable deformations during ventral flexion of the symphysis. The joint also permits 5–15° of dorsal flexion in most specimens.

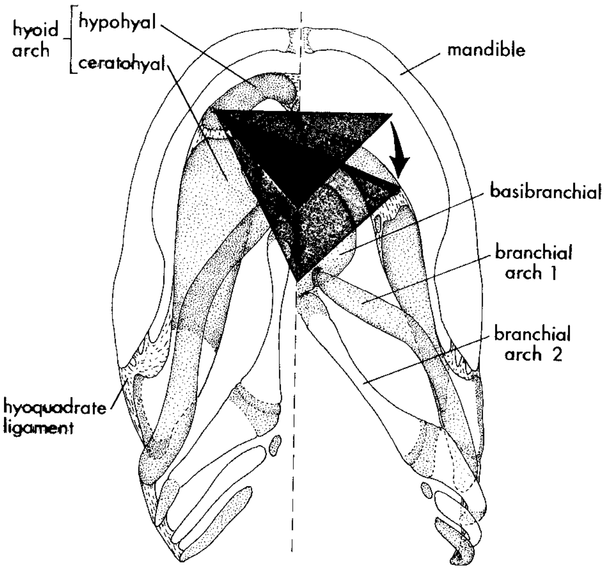

Figure 4. Ventral view of the mandibles and hyoid apparatus of *C. alleganiensis* to show the triangular arrangement of hyoid and branchial arch attachments. Relaxed condition is shown at left, asymmetrically abducted condition is shown at right. Abduction results in elongation of the triangle because the basibranchial is loosely attached to the ventral hyoid arch elements, whose forms and attachments, in turn, also allow some spreading of the base of the triangle.

minutes manipulating prey prior to swallowing. All animals switched to a different pattern of jaw and hyoid movements during swallowing.

The mandibular symphysis in *Cryptobranchus* is highly mobile (max. angular displacement = 45–70°). Unlike many species of salamanders, the dentaries of *Cryptobranchus* are separated by two pads of elastic cartilage (fig. 3). The larger ventral pad fills a triangular space between the dentaries and is compressible, even in preserved specimens. In combination with the mobility of the quadrate-articular joint between the mandibles and the skull, the arrangement of these cartilages and the surrounding symphyseal ligaments permits about 40° of ventral flexion but limits dorsal flexion to 5–15° in most specimens. In one specimen, the symphysis flexed 55° dorsally but only 15° ventrally.

In *Cryptobranchus*, asymmetric movements of the hyoid, if viewed as rotation around the longitudinal axis, approach 80° (approximately 40° to either side of the horizontal plane). The triangular arrangement of movable attachments between the median and paired elements (fig. 4) permits considerable independent motion of the distal ends of the left and right ceratohyals. Despite slight structural differences[15], the hyoid of *Andrias* also occasionally displays asymmetry in its movements.

Discussion. The structure and behavior of the feeding apparatus in *Cryptobranchus* and *Andrias* are unique among previously studied suction-feeding vertebrates. However, during reduction or transport of prey, the heads of various fishes[16, 17], reptiles[18–20], and mammals[21, 22] display bilaterally asymmetric or asynchronous patterns of bone movement that are generated by similarly asymmetric or asynchronous patterns of muscle activity. *Cryptobranchus* and *Andrias* are the first amphibians in which asymmetric jaw movements have been demonstrated. Whether the structural attributes of *Cryptobranchus* and *Andrias* which permit these movements are primitive or derived features for salamanders remains unclear although, on the basis of other characters, cryptobranchids are generally regarded as primitive salamanders[23]. On the other hand, the potential for bilateral asymmetry is probably a primitive feature of the control system of the vertebrate feeding apparatus because: 1) it appears in some representatives of virtually all major vertebrate clades, and 2) it seems functionally related to a simple property of feeding, which is that prey (or food) can rarely be expected to be symmetrical or to occupy positions that are bilaterally symmetrical with respect to the oral cavity. We suggest that in many vertebrate taxa, the nature of attachments between right and left skeletal elements severely limits kinematic asymmetry although the behavior of neural and muscular systems may display considerable asymmetry. As cautioned by Lauder and Norton[24], future electrophysiological studies of feeding in lower vertebrates should not assume bilateral symmetry in nervous or muscular activity.

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