Introduction

The retention of larval characters in otherwise sexually mature animals, neoteny, is found in all families of salamanders (Dent 1968; Duellman and Trueb 1986). On the basis of hyobranchial apparatus and skull morphology, Reilly (1986, 1987) found that the ontogeny of neoteny follows two basic patterns. In the family Ambystomatidae, for example, there is complete retention of larval characters for both gill structure and skull morphology, whereas in the Salamandridae, there is variable retention of larval characters in gill structures, but skull morphology is adult.

Regardless of which ontogenetic pattern is followed, for all neotenic forms sexual maturity is dependent on functional and mature reproductive structures. The cloacal anatomy has been described for a number of salamander species (Sever 1981, 1988a, 1991) and, in general, spermatophore deposition and pheromone production depend on the maturation of a variety of glands and accessory structures. Adults with structural abnormalities of the cloacal glands cannot produce spermatophores (Sever et al. 1989).

Larval features are retained completely in the nonreproductive tissues of neotenic Ambystoma gracile (Reilly 1987). For this reason, we compared the cloacal anatomy of metamorphosed and neotenic A. gracile to ascertain the degree of maturity in their reproductive structures and whether the timing of onset of gland development differs between the forms.

Materials and methods

Cloacal tissue from 16 A. gracile was examined histologically. A mature metamorphosed male (UMMZ 133156.2) of 97.8 mm snout to vent length (SVL) and a metamorphosed female (UMMZ 133156.1) of 95.6 mm SVL were collected in breeding condition on 17 March 1969 in Polk County, Oregon. Fourteen other A. gracile were collected from Hollyburn Mountain, West Vancouver, British Columbia, and Langley, British Columbia, on 7 July 1988. Those from Langley were collected as recently hatched, young-of-the-year larvae and those from Hollyburn Mountain hatched in the laboratory from naturally spawned egg masses. Larvae were raised individually in glass bowls with 1 L of aged dechlorinated water and maintained at 20°C on laboratory shelves in a room receiving subdued natural lighting (in Toronto, Ontario). Small larvae were fed Daphnia, and as they grew, they received Tubifex worms.

After metamorphosis, the transformed individuals were kept in glass bowls with damp paper towels at 20°C under the same laboratory conditions as individuals remaining branchiate. Metamorphosed animals were fed pieces of earthworm.

The ploidy of the 14 laboratory-reared animals had been determined by measurements of DNA quantity in erythrocyte nuclei made by means of flow cytometry: 13 were diploid and 1 was autotriploid (Lowcock and Licht 1990). Twelve diploid animals were killed on 21 August 1989 by prolonged immersion in tricaine methanesulfonate (MS 222). The autotriploid and one diploid were killed on 3 May 1989. Sexual maturity was assessed by the presence of enlarged testes and pigmented, coiled vasa deferentia in males and enlarged, pigmented ova in females (Semlitsch 1985). For comparison with the autotriploid, on 3 May 1989 three other neotenic males (75, 77, and 78 mm SVL) were examined for sexual maturity, but their cloacae were not examined histologically.

Based on the condition of the vasa deferentia, the autotriploid, a neotenic male (75 mm SVL), did not show any signs of sexual maturity. The other 13 individuals included 4 mature neotenic males (65, 72, 75, and 77 mm SVL), 3 mature metamorphosed males (74, 78, and 79 mm SVL), 3 mature neotenic females (63, 65, and 76 mm SVL), and 3 mature metamorphosed females (71, 78, and 80 mm SVL).

Individuals were preserved in 5% formalin (the two UMMZ specimens had been subsequently stored in 65% ethanol). Following removal of the cloacal region, the tissue was dehydrated in ethanol cleared in Histosol (National Diagnostics Inc., Manville, NJ), and embedded in paraffin, and 10-μm transverse sections were cut with a rotary microtome. Sections were stained with haematoxylin–eosin. Terminology for cloacal structures follows Sever (1981, 1988a, 1991).
Results

No differences were evident in cloacal anatomy among the captive-reared mature neotenic and metamorphosed males and females (Figs. 1–6). The cloacal glands of the wild-collected metamorphosed individuals in breeding condition (Figs. 3, 6) were more hypertrophied than those of the individuals raised in the laboratory (Figs. 1, 2, 4, 5). Overall, the cloacal glands of captive-reared mature individuals resembled those of mature individuals of other species examined outside the breeding season (Sever 1981, 1988a). The cloacal glands of the captive-reared mature males, however, seemed more developed than those of mature females from the same cohort. In the autotriploid male, cloacal glands were poorly developed and represented by cords of epithelial cells that can be regarded as little more than Anlagen (Figs. 7 and 8).

In the captive-reared mature females (both neotenic and metamorphosed), the dorsal glands, ventral glands, and spermathecae were distinct and recognizable, but no secretory product was evident in any of these glands. The dorsal glands, however, also possessed little secretory product in the mature female in breeding condition. Dorsal glands, when present, are usually rudimentary in salamanders and their function is unknown.
neotenic individuals is believed to be of adaptive value in providing more effective gill respiration and in the advantages of a unidirectional water flow feeding system (Reilly 1987; Lauder and Reilly 1988; Reilly and Lauder 1988). The genetic control for neoteny was thought to be a single recessive gene (Tomkins 1978), but recent evidence indicates that it is probably polygenic (Semlitsch and Wilbur 1989; Harris et al. 1990). In habitats where both metamorphosed and neotenic morphs coexist (Licht 1969, 1975; Eagleson 1976; Collins 1981), interbreeding would facilitate the genetic maintenance of both forms. In fact, Knudson (1960) reported courting between neotenic and metamorphosed A. gracile.

As indicated by the same level of development of the cloacal glands and the condition of the vasa deferentia in males and ovaries in females, the onset of sexual maturity does not differ in metamorphosed and neotenic forms. Semlitsch (1985) found that under natural conditions, metamorphosed and neotenic A. talpoideum appear to mature at the same time.

In the case of the autotriploid individual, undeveloped testes and vasa deferentia and the lack of mature cloacal glands support the finding of Fankhauser (1945) and Lowcock and Licht (1990) that triploid male salamanders, though normal in somatic growth, are slower to mature reproductively and most likely are functionally sterile.

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