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Short Communications

The regeneration of newt limbs deformed in nature

P.A. Tsonis[1] and G. Eguchi

Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya 464 (Japan), and Department of Developmental Biology, National Institute for Basic Biology, Myodaiji-cho, Okazaki 444 (Japan), 2 July 1984

Summary. The regeneration of abnormal limbs from a wild newt population was examined. The abnormalities were: mirror symmetrical reduplications of digit parts, deformed wrists, or limbs with supernumerary digits. Normal regeneration resulted after amputation of the abnormal parts, regardless of the original deformity. The results suggest that the abnormalities are probably the result of local trauma to the limbs.

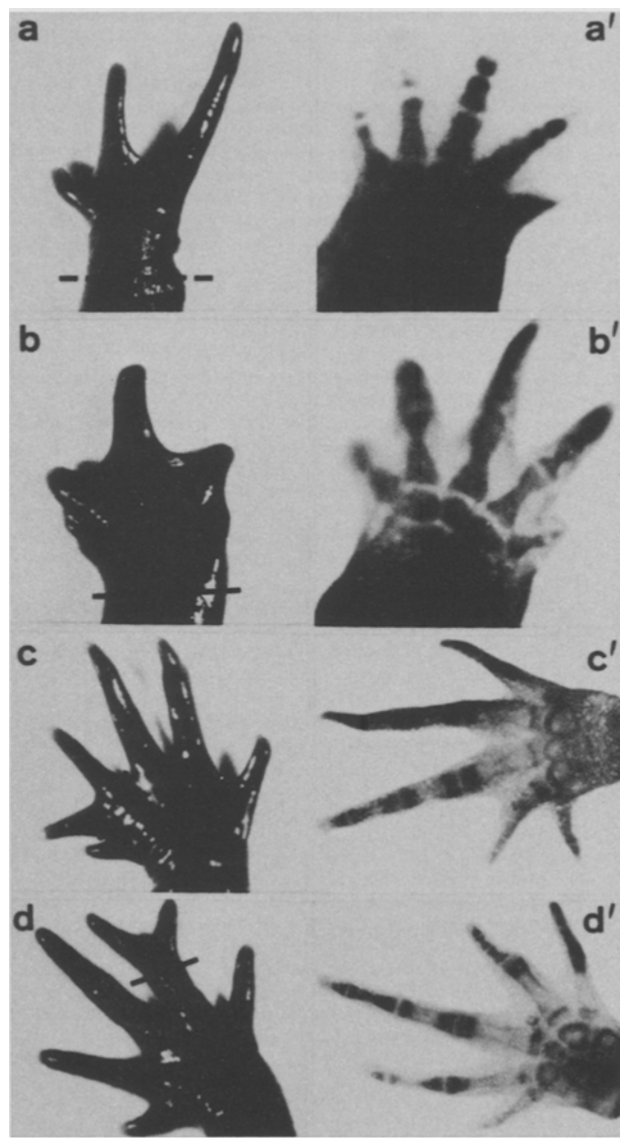
Key words. Newts, wild population; *Cynops pyrrhogaster;* limb regeneration; deformed limbs.

After a newt limb is amputated at any point along its axis, the process of regeneration begins, and a replica of the lost part is regenerated. The mechanism that controls this phenomenon is still obscure. According to the positional information theory, the blastema cells are ruled by positional information and, therefore, they can know (or recognize) their topological position in the limb. This is the most essential reason why only the distal parts to the amputation level can be regenerated[3,4]. Usually the regenerated limb is normal unless the blastema is subjected to certain mechanical or chemical treatments that affect the pattern formation process, and therefore the positional information[5-9]. We feel that studies on the regeneration of abnormal limbs will provide important information of the process of pattern formation. In addition, the information from the regeneration of such limbs may give clues on their origin. In the present study, we examined regeneration in wild newts with abnormal limbs at the time of capture. Despite the type of abnormality, the limbs were regenerated normally.

Materials and methods. Adult newt *Cynops pyrrhogaster* were collected in Tagarasu, Fukui prefecture, Japan. As the table summarizes, of the 11 cases tested, the abnormal limbs consisted of some with mirror symmetrical reduplication of digit parts (case 4, 5, 7, 10, 11), some with supernumerary digits (cases 2, 6, 8), and some with partially deformed wrists (cases 1, 3, 9). Only the abnormal parts of these limbs were removed except in case 2, in which a part was left. After a period ranging from 47 to 90 days, the regenerating limbs were excised, fixed in Bouins' solution, depigmented with 10% H_2O_2 and stained with Victoria blue B. Limbs were destained in 70% ethanol and cleared in methylbenzoate.

Results and discussion. The results appear in the table and the figure. As shown, regardless of the original deformity, the regenerated limbs were normal in all cases, except No. 2 in which the abnormal structure (supernumerary digit) had not been completely removed. Additionally, the accessory structure of case 8 did not regenerate. One of the two events probably accounts for the original abnormalities: 1) the abnormal limbs were regenerates produced after the newts' normal limbs became amputated or wounded in the wild; 2) the newts' limbs were deformed by disturbance of morphogenesis during embryonic development. If the first explanation is true, the present results confirm some experiments with abnormal hindlimbs from a wild population of *Rana* tadpoles[10], but are not in agreement with several other studies. Dearlove and Dresden[11] reported that repeated amputations give rise to increasingly abnormal regenerates. If the abnormal limbs used in the present study resulted from amputation in nature, then the subsequent amputation described here should have worsened the regenerates' deformities. To the contrary, regeneration was normal.

The literature also contains much contradiction concerning the regeneration of *induced* abnormal or polydactylous supernumerary limbs. Normal regeneration followed removal of



Regeneration of abnormal limbs. *a*: Case 1 limb with deformed wrist, × 4. *a'*: Case 1 regenerated with normal wrist having 5 digits, × 6. *b*: Case 3 limb with three digits connected with a lamina, × 4. *b'*: Regenerated case 3 limb with five normal digits, × 6. *c*: Case 7 limb with reduplication of II digit, × 3. *c'*: Case 7 limb with II digit regenerated normally, × 4. *d*: Case 11 limb with reduplication of IV digits, × 4. *d'*: Case 11 limb with IV digit regenerated normally, × 4. (× = magnification). Limbs were amputated at a level shown by dashed line.

Results of the regeneration of abnormal limbs in the adult newt *Cynops pyrrhogaster*

Case No.	Type of abnormality	Regeneration	Day of examination post-amputation
1 rhl	Deformed limb with abnormal tarsal region and 3 digits	Normal	90
2* lhl	6 digits	Identical	90
3 rhl	Limb with 3 digits connected with epidermal lamina	Normal	90
4 rhl	Mirror symmetrical supernumerary structures of II and III digits	Normal	67
5 rhl	Mirror symmetrical supernumerary structure of III digit	Normal	90
6 lhl	Extra digit	Normal	60
7 rhl	Mirror symmetrical supernumerary structure of II digit	Normal	90
8 lfl	Accessory digit-like structure at the metatarsal region	The accessory structure was not regenerated	90
9 rhl	Digits with defects in phalanges	Normal	90
10 lhl	Supernumerary structure of V digit	Normal	48
11 rhl	Mirror symmetrical supernumerary structure of IV digit	Normal	90

*The supernumerary digit was not completely removed. rhl: right hind limb; lhl: left hind limb; lfl left fore limb.

such limbs induced in the flank of *Triturus* larvae by homoplastic grafts of nasal placode[12], by grafting limb bud tissues[13], or by treatment with carcinogens[14]. Yet, Slack and Savage[15] reported that the amputation of mirror symmetrical limbs produced in the axolotls by transplantation of the rudimentary limbs gave rise to mirror symmetrical limbs in 66% of the cases. However, the abnormalities of the limbs used in this study were not induced by transplantation, but their cause is rather enigmatic. Moreover, in this study even unusually abnormal limbs regenerated normally, and amputation of mirror symmetrical parts of the digits (cases 4, 5, 7, 10, 11) gave rise to normal single digits. If the second explanation is true, the present data suggest that the regulatory factor(s) in pattern formation were intact when structural disturbance occurred during development of the embryo or limb.

Whatever the cause of the abnormalities, the present results show very clearly that when amputation is performed proximal to an abnormal structure, normal regeneration results. The regenerates reflect the structural patterns of the limb stump which, in the present study, was normal. This suggests that the abnormalities are a result of local disturbance during blastema formation, or a trauma in the intact limb which may effect the process of pattern formation.

We believe that experiments with abnormal limbs will continue to supplement information on how positional information operates. One question to be addressed is, how does regeneration of abnormal limbs (not supernumerary structures) take place when a part of the abnormal limb is left? Will the same type of abnormality be regenerated? Here, carcinogen-induced abnormal limbs can be used[6]. Such experiments will give information for a deeper understanding of the mechanisms underlying pattern formation.

1 Present address: The Cancer Res. Center, La Jolla Cancer Res. Foundation, 10901 North Torrey Pines Rd, La Jolla, California, 92037, USA.

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The conduction velocity, number and diameter of unmyelinated fibers in Remak's nerve

J.P. Hodgkiss and H.M. McIlroy

Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS (Scotland), 30 July 1984

Summary. Remak's nerve in the chicken was examined ultrastructurally and electrophysiologically to determine the characteristics of fibers in the nerve trunk. The ratio of unmyelinated fibers to myelinated ones was 111:1. The mean number of unmyelinated fibers was 3555 ± 232 (SEM, n = 5) and they had a mean diameter of 0.502 ± 0.034 (SEM) µm. The compound action potential consisted almost entirely of a large diphasic waveform which had a mean peak conduction velocity of 0.62 ± 0.031 (SEM, n = 5) $m \cdot s^{-1}$ at 37°C.
Key words. Chicken; conduction velocity; Remak's nerve; unmyelinated fiber diameter.

Remak's nerve (the intestinal nerve) is an autonomic ganglionated nerve trunk which has no mammalian homologue. The available evidence suggests that it contains ascending efferent fibers[1], ascending and descending adrenergic fibers[2,3], and afferent fibers from enteric cholinergic neurones[4]. In this study the fiber composition and the spectrum of fiber diameters in the nerve trunk have been determined using ultrastructural techniques. In addition the peak conduction velocities of unmyelinated fibers in the nerve have been determined from the compound action potential.