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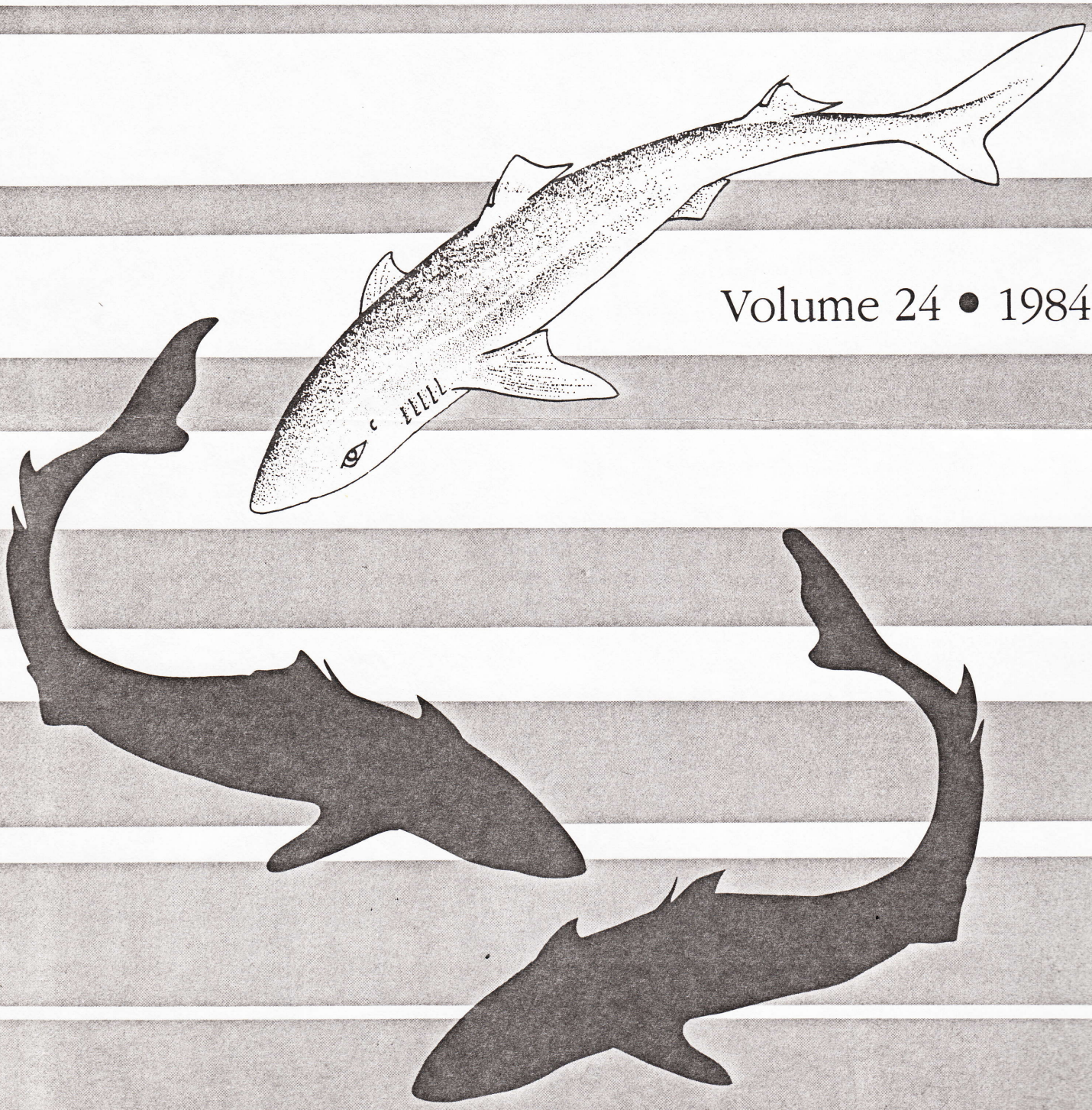

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SKELETAL PATTERN VARIABILITY IN NATIVE AND REGENERATED LIMBS OF THE RED-BACKED SALAMANDER, PLETHODON CINEREUS

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The plethodontid genus Plethodon is considered to be morphologically conservative (Highton, Evol. Biol. 10:397-436, 1977), especially with regard to skeletal variation between and within species (Wake, J. Morphol 113:77-118, 1963). However, a recent survey of a northwestern Nova Scotia population of Plethodon cinereus by Hanken (Can. J. Zool. 61: 1925-1931, 1983) revealed a remarkably high incidence of limb skeletal variants which includes nine different carpal and five different tarsal patterns as well as reduced phalangeal formulae in some digits. My own recent investigations on patterning during limb regeneration in this species warranted the following study to determine 1.) the relative variability of mesopodial (carpal and tarsal) patterning in native limbs of the MDI population of P. cinereus and 2.) the fidelity with which limb skeletal variants are regenerated, after limb amputation, as a measure of genetic control in epimorphic patterning.

Adult red-backed salamanders collected on Mount Desert Island were immobilized by immersion in 1% MS 222 and subsequently placed on the stage of a dissecting microscope for preliminary limb evaluation and amputation. One hundred-sixty forelimbs and 74 hindlimbs were included in this aspect of the study. Limbs were evaluated for superficial morphology, especially number of digits, and then amputated just distal to either elbow or knee. The distal segments were fixed in neutral buffered formalin for at least two days before being processed by a dichromic skeletal staining technique (Alcian blue/Alizarin red) according to the procedure adopted by Hanken and Wassersug (Funct. Photog. 16: 22-26,44, 1981). The animals were maintained as described previously (Bull MDIBL 17: 2-3, 1977) for 60-90 days to allow full limb regenerate morphogenesis and histogenesis. At the time of sacrifice the animals were again immobilized with MS 222, the regenerates evaluated superficially for number of digits per limb and then exarticulated at either shoulder or hip girdle. Entire limbs were, again, fixed and whole mount stained as above. To eliminate bias in comparing native limb skeletal patterns with matched regenerates, the cleared and stained limbs were coded (by C.E.D) before detailed analysis was done on the mesopodial patterns (by J.H.). The standard and currently known variant skeletal patterns with which these limbs were compared and catagorized are well-documented (Hanken, op. cit.).

The typical carpal pattern for P. cinereus consists of eight separate and named skeletal elements (Hanken's pattern #I, op. cit.). Eighty-six percent of the island population's forelimbs were of this type. Variations about this norm (patterns #II-VIII) are the result of specific interelement fusions. In the MDI population, three different fusion combinations were found in low

(<10%) to very low (1%) frequency. The two more common variations, patterns II (6%) and V (8%), are the variants that also predominate in both the Nova Scotia and a Virginia population, yet the relative frequencies of these shared patterns, while remarkably similar in the Maine and Virginia populations, are strikingly different in the Nova Scotia population (Hanken and Dinsmore, J. Herpetol., in press). These data provide evidence that the MDI population of P. cinereus has a typically conservative limb skeletal morphology and underscores the unusually high variability of the neighboring Nova Scotia population. Hindlimbs show less variability, expressing only a single fusion combination, but support the conclusions drawn from the carpal data that the Nova Scotia population is unique in its higher limb skeletal variability and frequency of variants.

When the regenerated limbs are matched with their native precursors and skeletal patterns compared, no congruence is found. The typical variants of native limb carpal fusions, i.e. distal carpal 4 with centrale or intermedium with ulnare, are not present in any of the regenerates. Nevertheless, there is a regular, extensive and non-random reduction of carpal elements through fusion (or non-separation during histogenesis) in most (92%) of the forelimb regenerates. And, again, the hindlimb regenerates reflect a similar rate (93%) though less pronounced variability in the pattern of tarsal fusions. In addition, the regenerates show a reduction in the number of digits replaced; an average of 3.65 digits for forelimbs and 4.47 digits for hindlimbs. Forelimbs of P. cinereus normally have four digits; hindlimbs, five. A reduction in the number of phalangeal elements per regenerated digit is also common. What emerges from this aspect of the study is a picture of epimorphic replacement of lost limb structures which is less-than-perfect yet with a predictable pattern of reductions in the skeletal pattern, the details of which are currently being evaluated. There appears at this time to be no correlation between the native limb skeletal pattern, either normal or variant, and that of its replacement in the regenerated limb.