

A Postfemoral Spot in *Plethodon*

ALBERT E. REYNOLDS, DePauw University

This paper is to report the occurrence of, and to present a preliminary description of, a morphological feature exhibited by certain salamanders of the genus *Plethodon*. Initiation of an investigation on inter-relationships among certain *Plethodons* was begun in the summer of 1956 by intensive study of live but chloretone-anesthetized specimens of *Plethodon jordani melaventris*. The author was up to that time acquainted with *P. j. melaventris* only by reading in the literature, and was both unprepared and surprised to observe that these animals exhibited a small oval area of lighter color located postero-dorsal to the junction of the thigh and trunk. Characterized by Pope and Hairston (10) as "immaculate . . . with a black belly," this salamander is so completely black that any pigment-free area, or any significant reduction in pigmentation intensity, is strikingly evident. This lighter oval area was first noted macroscopically in animals swimming about in finger bowls of dilute chloretone during the anesthetization period. Its consistency of appearance led to its being recorded on data sheets, and for conciseness it was termed a "postfemoral spot."

This report is based on factual data which have been derived from specimens collected in central Indiana and also in the Southern Appalachians where the Highlands Biological Station, Highlands, North Carolina, was the base of operations, and includes specimens accumulated over five warm-weather seasons (1956-1960, inclusive). During the summers of 1956 and 1957 the work was aided by National Science Foundation grants administered by the Highlands Biological Station. Administered by DePauw University, continued work in the Southern Appalachians was aided by grants from the Eli Lilly Company and the Esso Educational Foundation in the summers, respectively, of 1958 and 1959. A grant from the Research Fund of the Indiana Academy of Science provided certain materials used in the histological preparations. For guidance and helpful advice, grateful acknowledgement is made to Professor Thelma Howell, Executive Director of the Highlands Biological Station, to Mr. Arthur Stupka, Naturalist, Great Smoky Mountain National Park, and to Mrs. C. P. Hickman at DePauw for photomicrographic assistance.

Description and Occurrence of the Postfemoral Spot

Plethodon jordani melaventris

The postfemoral spot illustrated in Figure 1 indicates the general size and shape of the spot in relation to its anatomical context. The longer dimensions were around 1.5 to 1.8 mm, the lesser dimensions or greatest widths were about 0.5 to 0.6 mm, and the spot was uniformly ovoid. The spots of different specimens were not uniform, but varied in vividness or degree of lightness as compared to the black of surrounding skin.

Under magnification by the binocular dissecting microscope (10X and 30X), any "normal" or "typical" skin of the posterior dorsal or dorsolateral area presented the visual impression of solid black interrupted by small white areas which represented the conical, tapering,

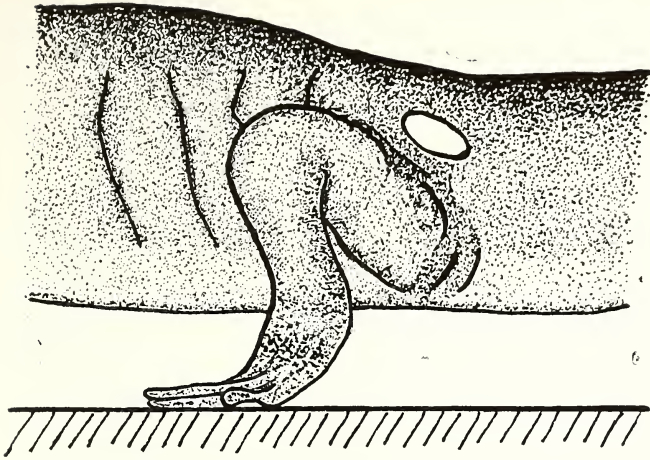


Figure 1. Semi-diagrammatic representation of the left side of *P. j. melaventris* No. 167 at pelvic level. Deliberately over-emphasized, the postfemoral spot is shown as an oval white area postero-dorsal to the junction of thigh and trunk (x 3, approximately).

superficial ends of flesh-colored skin glands. The black, closely-compacted melanophores appeared to be right at the surface, but if the skin were touched with a probe or teasing needle and either depressed or wrinkled, it became apparent that the black-containing layer was overlaid by a thin epidermis of almost perfect transparency. Within the oval area of the postfemoral spot, however, the melanophores were very deep within the skin, and were overlaid by a correspondingly thickened layer of clear tissue. Examination along the edges of the spot clearly revealed the melanophores sloping downward as they receded from a superficial to a deeper and more basal position within the dermis; these edges also revealed clearly the compensatory thickening of the overlying clear tissue as the melanophores sloped downward to the deeper position. While translucent to the point of almost complete transparency, under certain angles of observation and certain conditions of lighting, this clear overlying tissue seemed to have a slightly bluish or grayish hue which gave it a glazed or sheen-like appearance.

In addition to the deep and basal position, the melanophores within the spot area were much less closely-compacted, less densely concentrated, than was the case in adjacent typical skin. This reduction in melanophore concentration was, as to degree, readily correlated with the variations in vividness or degree of lightness of postfemoral spots mentioned above. In some of the postfemoral spots which were most vivid and easily detected by the naked eye, only a few scattered and isolated melanophores could be seen deep in the dermis with the aid of the binocular microscope.

To get some clue as to the underlying basis of the postfemoral spot in terms of skin organization, extension of the study into the realm of the histological was undertaken. The tissue excised included the postfemoral spot plus a small expanse of surrounding typical skin. Spread

on a small piece of cardboard to prevent curling, this was plunged into Bouins fluid for fixation. Imbedded in paraffin and sectioned at 8 microns, the tissues were stained in a combination of Delafield's hematoxylin and Mallory's triple stain, and finally mounted in balsam. Due to the preliminary nature of this phase of the investigation, it is not yet possible to give statistical results, so descriptive comment will be made on an average case.

From one such average case, Figure 2 shows a cross-section which included the postfemoral spot and adjacent non-spot skin. The latter, in

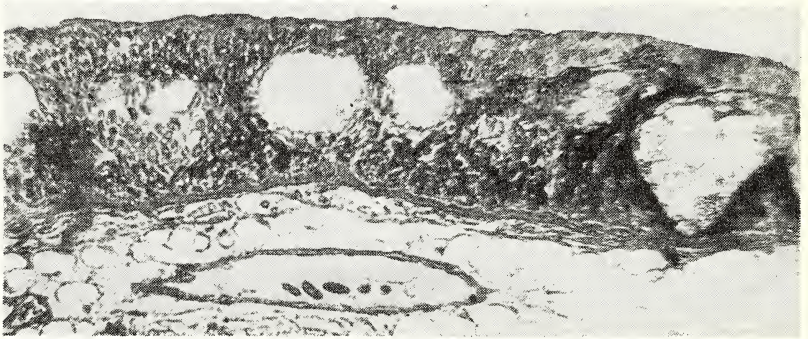


Figure 2. Section of postfemoral skin from *P. j. melaventris* No. 138 showing about half the postfemoral spot and some typical non-spot skin at the right. On the right, note heart-shaped granular gland in non-spot skin with thickly-clustered melanophores occupying most of dermal thickness. Note downward slope of melanophores to the thin and fragmentary occurrence in the spot; a small cluster of melanophores occurs to lower left of largest mucous gland near center of figure. Note thickenings of epidermis between mucous glands. Most basal layer of dermis is compact dermis consisting of wavy parallel connective tissue fibers.

this specimen, measured about 10 to 11.2 microns in thickness of which 1.4 to 2.2 microns was attributable to epidermis, 8.6 to 9.0 microns to dermis. Judged by the nuclear count, the epidermis was three to five cells thick, organized as a stratified epithelium. Just beneath it occurred, in non-spot skin, the spongy layer of the dermis; this included scattered connective tissue fibers randomly oriented, numerous nuclei, acini of mucous and granular glands, and melanophores. Occupying most of the dermal thickness, these melanophores were very numerous and overlapped each other; in general melanophores occurred from the lower epidermal border down into the dermis to a level about 1.2 microns above the basal border of the dermis. This deep 1.2 microns of melanophore-free dermis consisted of a small portion of the spongy dermis and all of the compact dermis which was made up largely of connective tissue fibers oriented parallel to each other and to the skin surface.

In the postfemoral spot, the middle of Figure 2 indicates that the melanophores were reduced to a fragmentary layer of deeply-situated and scattered melanophores above which occurred the clear overlying tissues that were described above from surface observation as being thickened in a compensatory manner. These can be seen to consist of

dermal elements devoid of melanophores plus an epidermis somewhat thickened except just above skin glands; the stretches of thickened epidermis usually measured 3.6 to 4.0 microns, and one measurement of 7.6 microns has been noted. No granular glands have as yet been observed in the postfemoral spot, though mucous glands occurred there to a normal extent. The downward slope of melanophores at the postfemoral spot edge is evident in Figure 2 which also indicates that what was described above as a recession to deeper location was not only that but also a drastic reduction of dermal thickness occupied by melanophores.

The *Plethodon jordani* Complex

The above data on *P. j. melaventris* represent observations on representatives from one of seven populations currently recognized as subspecies of *Plethodon jordani* (Schmidt, 11). The postfemoral spot has been seen in 92 specimens of *melaventris* collected from a varied sampling of its range. In addition, 14 live specimens on hand as of this writing clearly evince the postfemoral spot to macroscopic observation. In this total of 106 animals, all observed alive, young stages as well as adults of both sexes are represented.

Collecting in the Southern Appalachians was accomplished along certain transects which extended through the range of *P. j. melaventris* and on into the ranges of some of the adjacent populations. The number of specimens studied from each of these adjacent populations was, of course, considerably less than the number of *melaventris* reported on above. Data sheets on eight specimens of the red-legged *P. j. shermani* contained descriptions of the postfemoral spot, and in addition two live specimens currently on hand exhibit the spot to the naked eye. Records on 17 specimens of *P. j. rabunensis* describe the spot as also do records on four specimens of the nominate population, the red-cheeked *P. j. jordani* of the Great Smoky Mountains. In the small numbers of these near-relatives of *melaventris* that have been studied, the postfemoral spot agreed so closely with the spot of that form that no further description is necessary.

Plethodon glutinosus

This species, the slimy salamander, is widely distributed over a range that encompasses most of the eastern half of the United States (Dunn, 3; Bishop, 2) hence it not only occurs in Indiana but also overlaps geographically and in some cases altitudinally (Hairston, 6, 8) the mountain-dwelling and restricted-range members of the *P. jordani* complex of the Southern Appalachians. In the latter area, use of the transect method involved collection at lower altitudes and the taking of specimens of *glutinosus*.

Careful comparison of one dozen cases from central Indiana with an equal number of *glutinosus* from the Southern Appalachians revealed no essential differences with respect to manifestation of the postfemoral spot, at least as seen in surface view; no histologic preparations on *glutinosus* skin have as yet been completed. In this species the postfemoral spot is not visible to the naked eye, the reason becoming apparent when the specimens were observed under magnification. There was no reduction of melanophoric concentration within the spot area; melanophores appeared to be as closely-compacted in the spot as elsewhere in surrounding

skin. However, the spot area could be identified as an oval area wherein the melanophores receded to a more basal and deeper position, and wherein there was compensatory thickening of the clear overlying tissue. From both collection areas typical spot measurements were 1.6 x 0.9 mm, 2.1 x 0.9 mm, and 1.4 x 0.6 mm in adult specimens. In some cases the recession to deeper location involved not only melanophores but also the glistening, crystalline-appearing, dead-white spots made up of iridophores which are so characteristic of *P. glutinosus*.

Small *Plethodons*

In addition to the large salamander just discussed, *Plethodon cinereus* and *Plethodon dorsalis* are species of small salamanders which also occur in Indiana. As compared to the predominant black of the former, these two small species have brown melanophores and also exhibit more variegated colors since they have white, red, and yellow chromatophores as well as some pigment-free spots of flesh color.

In neither of these small *Plethodons* could a postfemoral spot be readily recognized, yet under magnifications of 10x and 30x certain aspects of the spot pattern were clearly evident. Live *dorsalis* exhibited an oval area corresponding to the spot location wherein the melanophores sloped off to a deeper position in a very characteristic manner, and wherein the clear superficial tissues correspondingly thickened. In most of these salamanders there occurred in the spot area a number of single, isolated, white iridophoric flecks which lay deep in the overlying clear tissue yet superficial to the deepened melanophores. In *dorsalis* there was furthermore a very slight, yet detectable, reduction in concentration of these deep postfemoral spot melanophores; in *cinereus*, on the other hand, there was no such reduction of concentration. Otherwise the *cinereus* spot was much like that of *dorsalis* save in these respects: (a) instead of isolated white flecks, whole spots made up of massed white chromatophores were involved in the chromatophoric retreat to deeper position, and (b) in the *cinereus* spot area there occurred a number of large capillaries which gave the area a slightly pink hue.

Discussion

In its most diagrammatic form, as seen in the skin of *P. j. melaventris*, the unique and distinctive characteristics of the postfemoral spot would include: (1) restriction of the chromatophores to a deeply recessed position basally in the dermis; (2) overlying the recessed chromatophores, a correspondingly thickened layer of clear and nearly transparent tissue; (3) reduction of compactness or concentration in the deeply-located chromatophores. The significance of this morphologic pattern is unclear; whether the postfemoral spot is in any way correlated with skeletal or muscular conditions in its anatomic area, or whether indeed it has any adaptive significance at all, must await more data and further study. However, the data presented above have made a reasonably strong case for the occurrence of this diagrammatic pattern or some modification of it in no less than three species of *Plethodon* plus four subspecies of a fourth species. This prevalence of occurrence might be interpreted as prima facie evidence for genetic control of the spot, and this in turn raises the interesting speculation that the variations shown to exist in

manifestation of the spot might represent restrictions on free gene flow between populations.

It may be noteworthy here to recall that the red-legged salamander of the Nantahala Mountains was originally described in 1906 as *Plethodon shermani* (Dunn, 3), and that it retained specific status for three and one-half decades until Bishop (1) relegated it to a subspecies as *Plethodon glutinosus shermani* (see also Bishop, 2). Rejected by Grobman (4), this relationship between *shermani* and *glutinosus* was also denied by Hairston and Pope (5) and Hairston (7), but the current view by which *shermani* constitutes one of seven subspecies of *P. jordani* has been challenged (Mittleman, 9). There furthermore exists admitted uncertainty concerning the true relationships between *glutinosus* and the members of the *jordani* complex, particularly with respect to *shermani* (Hairston, 7). It is this type of uncertainty which renders most intriguing any morphological situation which gives promise of shedding light on the problem of gene flow and possible variations therein, which additional study of the postfemoral spot might very well do.

Summary

The postfemoral spot is an oval patch of skin located postero-dorsal to the junction of the thigh and trunk which is characterized by (1) reduction of melanophoric concentration, (2) recession of such melanophores as do exist in the spot to a deep dermal position, (3) overlying the deepened melanophores, correspondingly thickened clear and pigment-free tissues made up of dermal elements and thickened epidermis. This spot is exhibited in its most diagrammatic form by *Plethodon jordani melaventrif* in which the spot has been seen in over 100 specimens and in which preliminary histologic studies have verified the characteristics observed from surface view. Limited studies on fewer specimens, and from surface examination only, indicate that a similar spot is characteristic of *P. j. shermani*, *P. j. rabunensis*, and *P. j. jordani*. *Plethodon glutinosus* from both the Southern Appalachians and central Indiana, as well as *P. cinereus* and *P. dorsalis* from the latter region, all exhibit modified forms of the diagrammatic postfemoral spot. The possible significance of this spot for speciative studies is discussed briefly.

Literature Cited

1. BISHOP, SHERMAN C. 1941. Notes on Salamanders with Descriptions of Several New Forms. Occ. Pap., Mus. Zool. Univ. Mich., No. 451, pp. 1-25.
2. BISHOP, SHERMAN C. 1947. Handbook of Salamanders. Comstock Publishing Company, Ithaca, New York.
3. DUNN, EMMETT REID. 1926. The Salamanders of the Family Plethodontidae. Smith College 50th Anniversary Publication, Northampton, Mass.
4. GROBMAN, ARNOLD B. 1944. The Distribution of the Salamanders of the Genus *Plethodon* in Eastern United States and Canada. Ann. N. Y. Acad. Sci. Vol. XLV, Art. 7, pp. 261-316.
5. HAIRSTON, NELSON G., and CLIFFORD H. POPE. 1948. Geographic Variation and Speciation in Appalachian Salamanders. Evolution 2: 266-278.
6. HAIRSTON, NELSON G. 1949. The Local Distribution and Ecology of the Plethodontid Salamanders of the Southern Appalachians. Ecol. Monogr. 19: 47-73.
7. HAIRSTON, NELSON G. 1950. Intergradation in Appalachian Salamanders of the Genus *Plethodon*. Copeia, 1950, No. 4, pp. 262-273.

8. HAIRSTON, NELSON G. 1951. Interspecies Competition and Its Probable Influence Upon the Vertical Distribution of Appalachian Salamanders of the Genus *Plethodon*. *Ecology* 32: 266-274.
9. MITTLEMAN, M. B. 1948. American Caudata. V. Notes on Certain Appalachian Salamanders of the Genus *Plethodon*. *Jour. Wash. Acad. Sci.* 38: 416-419.
10. POPE, CLIFFORD H., and NELSON G. HAIRSTON. 1948. Two New Subspecies of the Salamander, *Plethodon shermani*. *Copeia*, 1948, No. 2, pp. 106-107.
11. SCHMIDT, KARL P. 1953. A Check List of North American Amphibians and Reptiles. *Amer. Soc. Ich. and Herp.*