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ON THE EVOLUTION OF AN ORIENTAL EARTHWORM SPECIES, *PHERETIMA ANOMALA* MICHAELSEN 1907

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Most individuals of this aptly named species are referable to three formae, though large collections usually have contained one to several individuals regarded as intermediates. F. *centralis,* in most localities much the rarer of the three, differs significantly from the generic pattern only in location of the male pores on xx. F. *typica,* the first to be found, is athecal and additionally distinguished from *centralis* by extra pairs of testes and male funnels in v-ix, frequently even supernumerary gonads in those segments, as well as by extra pairs of mushroom-shaped glands in one or more of segments xxi-xxiv. F. *insolita* is thecal, each organ of the battery normal and the pores on 5/6-7/8 as in *centralis,* but lacks the mushroom glands and the male genital terminalia.

According to the first attempt at explaining the origin of these differences, f. *typica* and f. *insolita,* are respectively male and female secondarily evolved from the normally hermaphroditic *centralis.* In spite, however, of the presence of testes in unusually large numbers, sperm are not matured in the supposed males. On the contrary, sperm are produced by the supposed females even though discharge of the matured gametes from the body seems to be impossible.

According to a second explanation, the two divergent forms result from selective inhibition of secondary sex organs, during post-hatching development, by metabolic products released from two different types of protozoan parasites. Before an opportunity could be found to study results of activities of the parasites that

obviously are present in such large numbers in the aberrant forms of this and also other species of *Pheretima*, data from other sources began to indicate the necessity for another explanation.

P. anomala has now been found in several widely separated localities in India to which it has been transported from its proper home somewhere to the east of the Irrawaddy River. Only f. *typica* was secured at each of those localities. Presumably then, this form breeds true. This assumption is also supported by the incidence of the various formae in certain localities in Burma, as for instance in the Kamaungthwe River region of Tavoy district. In two collections from that area, taken two months apart, *centralis, typica, insolita* and the intermediates were represented by 0+0, 377+69, 11+2, and 31+8 specimens respectively. The intermediates, in most of these cases, would be referable to a less strictly defined *insolita* as they were distinguished only by the possession of one or more of the mushroom glands characteristic of *centralis* and *typica*.

F. *insolita* has not been found as yet in complete isolation, but its incidence is high in some localities. One collection from Taungyi, in which *centralis, typica, insolita* and intermediates were represented by 7, 32, 293 and 3 specimens respectively, also suggests a possibility of a true-breeding form. Here again the intermediates were referable to a less strictly defined *insolita*.

F. *centralis* never has been found in isolated colonies. It usually can be secured in most localities in Burma if search for it is continued long enough. Incidence always has been low, the greatest, according to the records now available, about 20 per cent, in a Karenni collection (from Koopra) in which the formae were represented, in order as above, by 21, 5, 67 and 9 specimens. Geographically, Karenni quite possibly is nearer the region where *anomala* may have arisen.

Throughout the Burmese portion of its range, *P. anomala* now seems to have been largely segregated into true-breeding forms. This could have been brought about, in earthworms, through replacement of sexual by parthenogenetic reproduction. As sperm are not produced by f. *typica* and since it lacks the organs for reception of male gametes from another individual, it can be assumed to reproduce parthenogenetically. In f. *insolita*, though sperm are produced, they cannot be discharged during copulation

and few individuals are likely to have an opportunity to copulate with those that can. No good evidence has been found for self fertilization. Presumably then, these supposed females, like the "males", usually do not reproduce sexually but parthenogenetically.

Evidence for copulation of individuals of different formae cannot now be presented due to destruction of the records during World War II. Copulation between individuals of *typica* certainly would appear to be futile. Cross copulation between individuals of *isolita* and *typica* would have no result for the *typica* worm, and the *insolita* partner ordinarily would receive into its spermathecae only secretions from the unusually well developed prostate glands of the *typica* individual. The opaque and non-iridescent coagulum often present in spermathecae of clitellate specimens of *insolita* may have been prostatic secretion received from a *typica* worm though the same non-iridescent opacity can eventuate from resorption of sperm in the seminal fluid received from another sort of partner.

Copulation in the cross *insolita* x *centralis* would not even result in transfer of prostatic secretions to the spermathecae of the normally sexual *centralis* individual which would then have to reproduce, if at all, by self fertilization (unlikely) or by parthenogenesis! In the latter case, offspring of the *centralis* type would be expected. The *insolita* partner, normally parthenogenetic, would however receive *centralis* sperm and unless parthenogenesis had become obligatory could be expected to reproduce sexually!

Copulation between two individuals of *centralis* can be expected to result only in *centralis* offspring but in view of the rarity of this form throughout most of Burma the cross can be expected there only infrequently.

A majority of the so-called intermediates resemble *insolita* except for the presence of one or more mushroom glands. Such anarsenosomphic worms can be expected to reproduce in the same way as *insolita*. The intermediates with male genital terminalia on one or both sides of the body can be expected to reproduce in the same way as *centralis*.

Of the 99 intermediates from the collections that were made throughout Burma during 1928-1932, 96 were assumed, because of their structure, to show relationships to both *insolita* and *cen-*

tralis, the only forms which can be expected, according to the preceding discussion, to produce offspring from a hybrid cross. Of the 96, 71 were without male terminalia like *insolita* and 25 had male terminalia as does *centralis*. This looks like a good approximation to the expected ratio in the F_2 generation if "*insolita*" is dominant to "*centralis*". The Mendelian relationship, if such it be, is however recognizable only because the mushroom glands of *centralis* have been carried over into the *insolita* phenotype. The *centralis* phenotype likewise was aberrant as prostates were variously located in segments xix-xxi, or only their ducts were present, or only one prostate was present, while mushroom glands always were lacking in xvii-xix and when present were in the segment where prostates should be expected.

The three remaining intermediates were thought to show relationships to *typica* and *centralis*. Possibly three of the 528 specimens that were identified as *typica* during the same period should also have been included. Each of these six worms had testes and male funnels in some or all of v-ix as in *typica* but three had a single normal *centralis* (or *insolita*) spermatheca and a fourth had a rudimentary one concealed within the parietes. The external aperture of one of the normal spermathecae was on 8/9 and this is the only instance that has been recognized in *anomala* of a pore at that posterior level. Offspring cannot be expected of a cross between *typica* and *centralis*, according to a previous portion of this discussion, and the mass of material collected subsequent to 1932 in the hope of clarifying the relationship was destroyed during the war. However, it can be said that an occasional individual of *typica* showed in seminal vesicles and/or testis sacs rather dubious evidence of having produced a few sperm. Hence, rarely, a cross between *typica* and *centralis* or even *insolita* may be possible. As the latter two are both thecal, presence of spermathecae would provide no clue as to which of the two forms had been involved in the hybrid parentage. If the hybridization is possible, the six cases just cited would seem to indicate that "*typica*", i.e., extra testes and male funnels, is dominant over "spermathecae" i.e., "*centralis*" or "*insolita*".

P. anomala must have arisen, presumably somewhere to the east or southeast of Burma, from an ancestor that may have had mushroom glands, or an equivalent, but which did have the male geni-

tal terminalia in xviii, as throughout most of the genus *Pheretima*. The evidence provided by aberrant individuals of this and other species agrees in indicating that transfer of capacity to develop prostate glands, from one segment to another, whether anteriorly or posteriorly, takes place in a single step rather than by the much more gradual sort of migration that has been assumed in the classical oligochaete phylogeny. An early, if not the first step, in the evolution of *anomala* was then the establishment of a mutation for transfer of prostate developing capacity from xviii to xx. No difficulty is to be expected for the male deferent ducts in reaching the new segment through which they must now open to the exterior, as numerous specimens of *insolita* have shown that the ducts can grow back as far as xxx behind which level male pores very rarely have been recorded in any family of earthworms. F. *insolita* also demonstrates that the male deferent ducts do not acquire an external aperture in absence of the prostates. Union of male deferent and prostatic ducts, regardless of the segment in which the glands are located, suggests that the former, on reaching the prostatic segment, are attracted towards the growing glands. Union presumably takes place at parietal level. Subsequently the prostatic duct ectal to the junction becomes much elongated, carrying the region of that junction deep into the coelomic cavity. In aberrant individuals without prostate glands but with well developed muscular prostatic ducts there is no distinct level of demarcation between the latter and the deferent duct. The gradual transition from one to the other suggests that premature union of the ectal end of the deferent duct with the ental end of the prostate rudiment, instead of at the side, may have had something to do with absence of the prostate gland.

Shortly after establishment of the translocation mutation, appearance of another one may be assumed, this time for permissive or facultative parthenogenesis. Due to the ability of a single individual to reproduce when a copulatory partner was unavailable, the species may have been able to colonize new areas much more rapidly than would have been possible otherwise. Certainly, *P. anomala* has spread through most of Burma, probably to a greater extent than any other species of the genus.

From the steady increase in number of earthworm species, in various families, that are being found to have uniparental repro-

duction, a parthenogenetic mutation appears to be fairly common. Though recognized in *anomala* hitherto only in association with additional mutations to be mentioned below, conditions in other species indicate that they can be independent of each other. Thus, in forms like *P. diffringens* (Baird) 1869, which is now widely spread throughout the United States, reproduction usually seems to be parthenogenetic in spite of presence of a complete battery of normal spermathecae and of normally developed male genital terminalia.

If now a third mutation, "athecal", inhibiting development of the spermathecae, were to arise in *anomala* at the appropriate time, establishment of a true-breeding line would be expected. As the athecal individual could not receive sperm from a copulatory partner, all of its own offspring would be mutants. Offspring of a normal copulatory partner would be in part athecal also and if the mutation is dominant its rapid spread through the population should be possible.

The athecal mutation also seems to be standard as the condition has appeared in individuals of species belonging to various families but with especially annoying frequency in the genus *Pheretima,* depriving the taxonomist of a whole set of the very organs most useful for his species identifications. The mutation has not been found, in other species of the genus, in association with extra testes. To obtain f. *typica* from a *centralis* ancestor, yet another mutation, "testis", is postulated. This too may prove to be standard as extra testes anterior to the usual gonad series already have been found in species of two other families. If this spawning of hypothetical mutations in a single line is to end here it is necessary to conclude that mutation "testis" is not of the apparently simple type of mutation "athecal". In addition to bringing about development of extra gonads and adding two or more pairs of mushroom glands to the *centralis* series it inhibits production of sperm by any of the gonads.

To get f. *insolita* from an ancestor like *centralis,* a mutation, "aprostatic", for inhibition of development of prostates, is required. This condition also appears throughout prostate-possessing families of earthworms but has been noticed more often in the genus *Pheretima.* Unless another mutation is to be postulated it must be assumed that "aprostatic" also expresses itself by

inhibition of development of the mushroom glands. For such an assumption a certain amount of evidence is provided by aberrant individuals in other species of the genus.

Mutations "athecal" and "aprostatic" have not yet been found in the same individual of *anomala* but the two conditions have appeared simultaneously in individuals of other species in the genus, thus depriving the sorely tried taxonomist of the last sets of organs required for species identifications. Association of the two mutations already has been found in at least one of the dozen species of *Pheretima* that have been accidentally introduced into this country.

Another hypothetical mutation, "aseptal", has long been established in *anomala*, as well as in many other species of the genus. This partially or completely aborts, during embryonic development or post-hatching growth, the transverse partition separating the coelomic cavities of segments viii and ix. Abortion rather than inhibition is indicated by persistent rudiments of varying size while incomplete or delayed penetrance is shown by occasional individuals in which a considerable portion of the septum is still recognizable or in which the partition even has become muscular. Yet another mutation, "uniporal", which has been involved in the ancestry of most species of *Pheretima*, results in union of the paired oviducts within the parietes so as to open to the exterior by a single, median pore. Here again, incomplete or delayed penetrance is suggested by the failure of the oviducts, in an occasional individual, to unite ectally thus resulting in a reversion to the ancestral condition with paired female pores.

Postulating a mutation capable of bringing about addition of five pairs of testes, along with five pairs of male deferent ducts — in one step — can be avoided in two ways at least. One alternative would be to assume that a mutation for addition of one or two pairs of testes at the anterior end of the existing series was repeated an appropriate number of times. Repetition of four different one-step-at-a-time mutations now seems to be required in the phylogeny of another genus currently under examination. Another alternative is provided by the widely accepted evolution of the Oligochaeta from marine Polychaeta during which diffuse germinal tissue was strictly concentrated into one intrasegmental location, within the septa close to the nerve cord, but in several

consecutive segments. Nine or ten pairs of gonads almost seem to be required in oligochaete phylogeny by the families of Microdrili. Anlage of such gonads laid down early in embryonic development of *anomala,* instead of being aborted throughout most of the series as usual, could be permitted by mutation "testis" to develop into a macroscopic discoidal stage. Origin of supernumerary gonads in some of the segments can be attributed to early embryonic fragmentation such as breaks a first nephridial rudiment into early components of the enteronephric excretory system in this same genus. Development of male funnels is however induced by the adjacent growing gonads. If the induction effect is adequate, anlage not only become plicate funnels but develop ducts which may even become continuous with those of the posterior segments.

Summary

Evolution of an advanced species from a more generalized generic type and segregation within that species of three true-breeding formae is attributed to the establishment of single effect and multiple effect mutations. Each of these postulated mutations is of a standard sort, required by conditions in aberrant individuals appearing in species of several genera, or by phyletic developments that obviously have taken place in genera of different families.