Cytogenetic Aspects of Evolution of the Family Heteroderidae[1]

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Cytotaxonomy implies the utilization of cytological information in taxonomy. Such information is mostly limited to the description of the karyotype, *i.e.*, chromosome number, morphology, size, sex chromosomes, presence or absence of supernumerary or other types of chromosomes, and the study of the behavior of the chromosomes during the mitotic and the meiotic cycle. With the adoption of the biological species concept in animal taxonomy, however, it became necessary for species delineation to know the mode of reproduction of a particular organism and its capabilities for effective interbreeding with related organisms. To meet the new requirements, cytotaxonomy extended its activities to include the study of the mode of reproduction through karyological analysis of gametogenesis, and the study of the behavior of the chromosomes in hybrids between related organisms. These new approaches are both cytological and genetic in nature. Therefore, in a broader definition, cytotaxonomy utilizes "cytogenetic" information for elucidation of taxonomic problems.

Analysis of the karyotype has been valuable in the study of the relationships within several animal groups such as primates, insects, rodents, etc. Karyotype comparisons have often permitted interpretations of the phylogenetic relationships of the members of a group and have indicated the direction of their evolution. The greatest phylogenetic interpretation value of the karyotype is often at the genus, and usually at or below the species level; this is because the causal relationships of the observed karyotypic variations are easier to interpret at the genus or species level due to a shorter evolutionary time scale compared to that of higher taxa.

In keeping with the overall objectives of this symposium, I should probably discuss in general terms the role cytogenetic information can play in solving problems in nematode taxonomy. A general discussion, however, involving all nematodes would be difficult to comprehend, and would provide no solutions to specific taxonomic problems. For this reason I will limit this discussion to the family Heteroderidae, an important family of plant-parasitic nematodes, in which a good deal of cytogenetic work has been conducted recently. General conclusions will undoubtedly be applicable to other families of nematodes with similar cytogenetic characteristics.

The central theme of this discussion is that evolution in the family Heteroderidae has been influenced by extensive modifications of the basic karyotype, including the establishment of polyploidy and aneuploidy, in association with the establishment of various types of parthenogenetic reproduction. By studying the karyotypic relationships of the present day forms of these organisms, and considering other available cytogenetic in-

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formation, we may be able to make some inferences as to the actual pathway of evolution of the family, and suggest a sound taxonomic treatment. Of course, evaluation of the cytogenetic information alone, to the exclusion of other available information, would share the same shortcomings of other taxonomic systems relying entirely on information of one kind. A better system of classification may result, if all information available with regard to cytogenetics, morphology, physiology, biochemistry, ecology, behavior and distribution of these organisms is considered and evaluated at the same time. In the present discussion emphasis will be placed on the following cytogenetic characters: karyotype, chromosomal behavior during gametogenesis, and mode of reproduction. Certain morphological and physiological characters will be considered in some cases.

According to the latest taxonomic treatment, the family Heteroderidae comprises five genera, *Heterodera* A. Schmidt, *Meloidogyne* Goeldi, *Meloidodera* Chitwood, Hannon & Esser, *Cryphodera* Colbran, and *Meloidoderita* Poghossian. A sixth genus, *Hypsoperine* Sledge & Golden, was synonymized with *Meloidogyne* in 1968 (15), but a new species described early in 1969 was placed in *Hypsoperine* as *H. ottersoni* Thorne (8). No cytogenetic work has been done with the monotypic *Cryphodera* and *Meloidoderita*, and therefore, these genera will not be included in the discussion.

Among the species of *Heterodera* studied thus far cytologically, 13 are diploid amphimictic with a haploid number of 9 chromosomes. These include *H. schachtii* A. Schmidt, *H. glycines* Ichinohe, *H. oryzae* Luc & Brizuela, *H. avenae* Wollenweber, *H. goettingiana* Liebscher, *H. cruciferae* Franklin, *H. carotae* Jones, *H. weissi* Steiner, *H. rostochiensis* Wollenweber, *H. tabacum* Lownsbery & Lownsbery, *H. virginiae* Miller & Gray, *H. mexicana* Campos, and Osborne's cyst nematode. The karyotype of all these species is quite similar. There are some differences in chromosome morphology, chromosome size, and chromosomal behavior during gametogenesis from species to species, but, in the absence of a detailed karyotypic analysis and comparison, such differences are of limited value in characterizing the species or suggesting relationships among them.

Another three species of *Heterodera* that have been studied cytologically, *i.e., H. trifolii* (Goffart), *H. galeopsidis* (Goffart), and *H. lespedezae* Golden & Cobb, as well as an undescribed species from *Rumex crispus* L., are morphologically closely related to *H. schachtii* and *H. glycines* and constitute a series of chromosomal forms, with somatic numbers ranging from 24 to 34, and reproduce by mitotic parthenogenesis (4). There is little doubt that these "species" have been derived from *H. schachtii* or *H. glycines* through various chromosomal changes leading to polyploidy and aneuploidy in association with the establishment of parthenogenetic reproduction (Table 1). It is very likely that all of them have evolved along the same phyletic line, or a number of parallel phyletic lines derived from the same basic species or group of related species, at different occasions and probably at different time periods. They constitute a parthenogenetic species complex completely separated reproductively and, therefore, genetically from the amphimictic species from which they have been derived.

From a taxonomic viewpoint, it is an open question whether members of such a parthenogenetic species complex should be treated as separate species (as has been done in the past in nematode taxonomy) without reference to their phylogenetic relationships, should be regarded as subspecies (14) or as properly designated, but unnamed, infra-

specific categories of the same parthenogenetic species. Nematode taxonomists need to find a practical solution to this problem, and at the same time to decide which of the theoretically numerous morphological and physiological variants within a parthenogenetic group should be recognized as separate taxonomic entities.

A similar case of parthenogenetic evolution in the genus *Heterodera* involves *H. oryzae* and *H. sacchari* Luc & Merny of the *H. schachtii* species group. These two species are very closely related morphologically and have the same geographical distribution. *H. oryzae* is a diploid amphimictic species with n = 9 chromosomes, whereas, *H. sacchari* has 2n = 27 and reproduces by mitotic parthenogenesis (6). It is very likely that *H. sacchari* has evolved from *H. oryzae*, or another amphimictic relative as a triploid parthenogenetic form (Table 1). *H. leuceilyma* Di Edwardo & Perry, is closely related morphologically to *H. sacchari*, has no males and undoubtedly reproduces by parthenogenesis. Although no cytological work has been done with *H. leuceilyma*, it very likely has evolved along the same parthenogenetic line with *H. sacchari* and, consequently, belongs to the same parthenogenetic species complex.

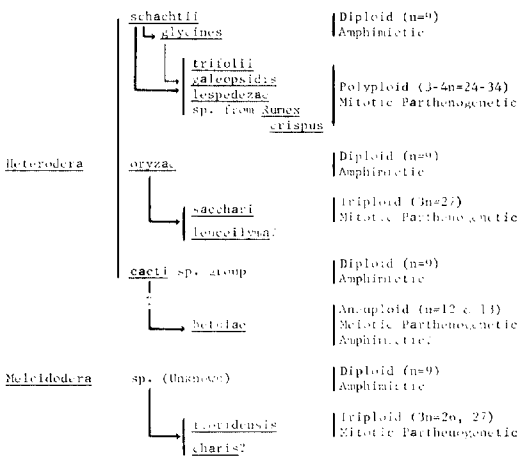
A third but different case of parthenogenetic evolution in the genus *Heterodera* includes *H. betulae* Hirschmann & Riggs (3) which reproduces by meiotic parthenogenesis and has a haploid number of 12 and 13 chromosomes (A. C. Triantaphyllou, unpublished data). *H. betulae* must have evolved from a diploid (n = 9) amphimictic relative, probably of the *H. cacti* Filipjev & Schuurmans Stekhoven species group, through a gradual increase of the basic chromosome number from 9 to 12 and 13, and the establishment of parthenogenetic reproduction (Table 1). The existence of individuals with 12 and 13 chromosomes in *H. betulae* indicates that the karyotype of this species has not yet stabilized. Furthermore, the occasional appearance of males, and the meiotic type of maturation of the oocytes suggest that reproduction by cross-fertilization may occur occasionally in this species. Consequently, the instability of the karyotype, and the facultative type of parthenogenesis, would tend to indicate that *H. betulae* has been derived recently and is still in a state of active evolution. This is in contrast to the evolutionary status of the *H. trifolii* species complex, in which the complete absence of males, and the obligatory type of mitotic parthenogenesis indicate an advanced state of evolution (although degenerative evolution), with no spectacular changes being expected in the future.

These, briefly, are the three separate lines of parthenogenetic evolution observed thus far in the genus *Heterodera*.

In the genus *Meloidodera*, the only species studied cytogenetically, *M. floridensis* Chitwood, Hannon & Esser, reproduces by mitotic parthenogenesis and has a somatic chromosome number of 26 and 27 (A. C. Triantaphyllou, unpublished data). Its chromosomes resemble those of mitotic parthenogenetic species of *Heterodera*, like *H. trifolii*, although they are smaller in size. *M. floridensis* can be regarded as a triploid parthenogenetic organism, probably derived from an amphimictic diploid ancestor with n = 9 chromosomes (Table 1). Further cytogenetic work may reveal the existence of some diploid amphimictic species of *Meloidodera* with n = 9 chromosomes, and thus confirm the previous assumption. *Meloidodera charis* Hopper, appears to be closely related morphologically and physiologically to *M. floridensis*, and also is suspected of being very similar cytogenetically. Apparently it belongs to the same parthenogenetic species complex as *M. floridensis*.

Turning our attention to the genus *Meloi-*

TABLE 1. Partial phylogenetic scheme, showing three separate lines of parthenogenetic evolution in the genus *Heterodera* and one in the genus *Meloidodera*.



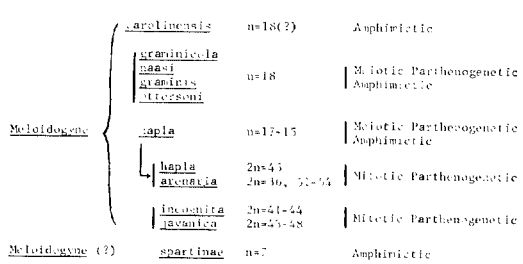
dogyne, we see that almost the entire genus has evolved along a parthenogenetic mode of reproduction. The only species that appears to reproduce exclusively by amphimixis is a new species that will be described as *M. carolinensis*, and which probably has 18 chromosomes (2) (Table 2). *M. carolinensis* can be considered cytogenetically as an ancestral species, representative of the central line of evolution of the genus. Its limited host range (it infects only blueberry and azalea) however, indicates a highly evolved parasite, at least with respect to host specialization.

The next most advanced species, from the standpoint of evolution of reproduction, are *M. graminicola* Golden & Birchfield, and *M. naasi* Franklin, which normally reproduce by meiotic parthenogenesis but under certain circumstances can also reproduce by amphimixis (12). They are closely related morphologically to *M. carolinensis* and have the same number (n = 18) of chromosomes, which can be regarded as the basic number for the genus *Meloidogyne*.

M. hapla Chitwood stands at the same level of evolution as *M. graminicola* and *M. naasi* from the standpoint of mode of reproduction, but it has gone a few steps further with regard to the evolution of its karyotype. The chromosomal complement of *M. hapla* has been reduced from 18 to 17, 16 and 15 (11). Moreover, some populations of *M. hapla* and all populations of *M. arenaria* (Neal) (10) have evolved further with the establishment of various degrees of polyploidy (2n = 45, and 36 or 51–54, respectively) in association with mitotic parthenogenesis. Probably these forms have been derived secondarily from populations of *M. hapla* through occasional fertilization of gametes of different chromosomal complement. Thus *M. hapla* with 45 chromosomes may have been derived through fertilization of an unreduced egg of *M. hapla* with 30 chromosomes by a reduced sperm containing the haploid set of 15 chromosomes.

M. incognita (Kofoid & White) and *M. javanica* (Treub) reproduce by mitotic parthenogenesis and are polyploids (2n = 41–44 and 43–48, respectively) (A. C. Triantaphyllou, unpublished data) and (9). Such forms are equivalent to the mitotic parthenogenetic populations of *M. hapla* and *M. arenaria* but, with our present knowledge, we cannot trace their phylogenetic derivation. Further search

TABLE 2. Species relationships in the genus *Meloidogyne* based on their karyotype and mode of reproduction.



will probably associate them with an amphimictic or, at least, meiotic parthenogenetic species of *Meloidogyne*.

Three species of the former genus *Hypsoperine*, recently synonymized with *Meloidogyne*, have been studied cytogenetically. Six populations of *M. graminis* (Sledge & Golden) of different origin have n = 18 chromosomes and reproduce regularly by meiotic parthenogenesis and occasionally by amphimixis (A. C. Triantaphyllou, unpublished data). Thus, *M. graminis* is cytogenetically similar with *M. graminicola* and *M. naasi*. Its recent transfer to the genus *Meloidogyne* (15) on the basis of morphological criteria is, therefore, supported by the available cytogenetic information. The recently described species *Hypsoperine ottersoni* is indistinguishable cytogenetically from *M. graminis* (A. C. Triantaphyllou, unpublished data) and may also be transferred to the genus *Meloidogyne* as *Meloidogyne ottersoni*, in the same species group with *M. graminicola* and *M. naasi* (Table 2). *Meloidogyne spartinae* (Rau & Fassuliotis), on the other hand, has a haploid number of 7 chromosomes—the smallest number observed in the family Heteroderidae—and reproduces by amphimixis (A. C. Triantaphyllou, unpublished data) and (1). It is difficult to speculate on its relationship with other *Meloidogyne* species. It can be assumed that, as an amphimictic species, with a chromosome number much different from the basic number of the genus *Meloidogyne*, *M. spartinae* is a basic species, and has not evolved from any of the *Meloidogyne* species studied cytogenetically thus far. Its cytogenetic characteristics together with existing substantial morphological and physiological differences would probably justify the assignment of *M. spartinae* to a separate genus.

Now let us discuss the possible relationships among the genera of the family Heteroderidae, and first the two main genera, *Heterodera* and *Meloidogyne*. Although considered to be taxonomically closely related, these genera are still quite far apart both morphologically and cytogenetically (13). The genus *Heterodera* has a basic chromosome number of 9 and predominantly amphimictic mode of reproduction. It may represent the main line of evolution from a hypothetical diploid (x = 9) amphimictic ancestor, and probably the only line of progressive evolution in the family Heteroderidae. The genus *Meloidogyne*, on the other hand, has a basic chromosome number of 18, and has followed a predominantly parthenogenetic line of evolution, which by its nature is a regressive or degenerative type of evolution. Several hypotheses have been expressed with regard to the relationship of the karyotypes of these two genera, but none has been satisfactory. In a recent analysis it was proposed that, until more evidence becomes available regarding the course of karyotypic evolution in each genus, the karyotypes of these two genera should be considered separately, without reference to their relationship (12).

Recent DNA measurements in hypodermal nuclei of second-stage larvae of various members of these genera confirmed the hypothesis that polyploidy exists within each genus, but failed to clarify the relationship of the karyotypes of the two genera (5).

The difficulty of establishing a definite relationship between these two karyotypes may actually indicate the lack of a close relationship between them. Therefore, if we postulate a common ancestor for the genera *Meloidogyne* and *Heterodera*, branching of the evolutionary lines that gave rise to these genera must have occurred in the very distant evolutionary past, so that characterization of such a common ancestor now, on a cytological basis, is almost impossible.

With regard to the genus *Meloidodera*, the type species *M. floridensis*, cannot be re-

garded as a direct phylogenetic link between the genera *Meloidogyne* and *Heterodera*, as has been suggested in the past on the basis of morphological characteristics, because it is a triploid parthenogenetic organism. As such, and because its karyotype is similar to that of *Heterodera, M. floridensis* can be regarded as the end product of an evolutionary line that originated quite early in the phylogenetic scheme from a diploid (n = 9) amphimictic organism, which was the hypothetical common ancestor of the genera *Meloidodera* and *Heterodera*. *M. floridensis* must have evolved slowly compared to the genus *Heterodera* due to its parthenogenetic mode of reproduction, which reduced the rate of genetic evolution. For this reason it has maintained some of the primitive characteristics of the common ancestor, such as the subequatorial position of the vulva, the presence of well-developed transverse cuticular annulations in the female, and the absence of a leathery brown, cyst stage.

This very briefly is an analysis of the phylogeny of the family Heteroderidae from a cytogenetic viewpoint. The most striking characteristic is the active evolution of the karyotype, and the establishment of polyploidy and aneuploidy in association with parthenogenetic reproduction. Expansion of the cytogenetic work to include other members, particularly the genera *Cryphodera* and *Meloidoderita* will be very useful.

The contribution of cytogenetics will be significant for many other families of plant-parasitic nematodes with similar cytogenetic characteristics as the family Heteroderidae. Recent cytogenetic work with various members of the genus *Pratylenchus* (7) has shown that evolution of this genus has been associated with changes of the basic chromosome number and the establishment of polyploidy and parthenogenesis. Some cytogenetic work has also been done with members of the subfamilies Hoplolaminae and Tylenchinae and sporadically with other plant-parasitic nematodes. It all supports the general conclusion that evolution of plant-parasitic nematodes has been influenced by extensive modifications of their karyotype and the establishment of various types of parthenogenetic reproduction. Consequently, cytogenetic analysis of various groups will undoubtedly yield valuable information for elucidating phylogenetic relationships and clarifying taxonomic problems.

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