single nucleotide change in 5*S* rRNA represents millions of years of evolution. Moreover, there is evidence in the fossil record for substantial morphologic discontinuity occurring in the molluscs, within relatively short geologic time spans (*15*).

Although microscopic examinations have indicated that the invertebrates we considered might harbor more than one type of symbiont (*16*), we recovered only one end-labeled, prokaryotic 5*S* rRNA from each. However, different 5*S* rRNA's vary in their efficiencies of end-labeling because of varying secondary structures at their termini, so it remained possible that other 5*S* rRNA's present in low abundance were not detected. This uncertainty was overcome by first digesting the mixed, unlabeled 5*S* rRNA population with ribonuclease T_1 or A, end-labeling the resultant oligonucleotides, and resolving the products by two-dimensional electrophoresis (Fig. 1D). Oligonucleotides are labeled uniformly with 5′-^{32}P, so that the relative radioactivities of species-specific oligonucleotides (defined by the RNA sequences) establish the 5*S* rRNA stoichiometries and are a test for the presence of unsuspected RNA's. Such analyses revealed no novel 5*S* rRNA's, and therefore we conclude that the symbiont populations in these animals are at least 90 percent homogeneous. In the case of the *Riftia* trophosome, we inspected material from two regions of the worm—the anterior one-third and the extreme posterior. The 5*S* rRNA's isolated from both segments were identical. By the criterion of 5*S* rRNA oligonucleotide content, presuming equivalent extraction efficiencies, the prokaryotic symbionts contribute more than 75 percent of the *Riftia* trophosomal ribosomes. The symbiont contributions to the *Calyptogena* and *Solemya* gill tissues are about 75 and 20 percent, respectively. In the one case examined (*Solemya*), no bacterial 5*S* rRNA could be extracted from nongill tissue.

The techniques used to characterize these two-component symbioses are applicable to more complex microbial populations as well. Isolation and analysis of rRNA's (or their genes) from diverse environments could be a powerful and general tool for the microbial ecologist.

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30. In evaluating the agreement of phylogenies with sequence data, it is assumed that all of the sequence positions are subject to independent base variation. Base-paired nucleotides are the most obvious exceptions to this assumption; if one nucleotide is known, its pairing partner can be predicted reliably. We have accounted for this redundancy by decreasing the weighting of paired positions to half that of unpaired positions. This data treatment results in more self-consistent phylogenies; that is, a better overall agreement between the observed pairwise distances and those implicit in the "optimal" trees.
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Chromosome Organization and Heterochromatin Elimination in *Parascaris*

Abstract. *A cytological analysis by modern banding techniques of gonial metaphases in two* Parascaris *forms that have been considered varieties but now seem to be two species* [P. univalens *(karyotype* 2n = *2) and* P. equorum *(karyotype* 2n = *4)] reveals a different chromosome organization in each.* Parascaris univalens *chromosomes contain only terminal heterochromatin, while* P. equorum *chromosomes also contain intercalary heterochromatin. In the somatic cells of both species during early embryogenesis, chromatin diminution occurs in and consists of the elimination of all heterochromatin independent of its localization in the chromosomes.*

Past studies on the nematode *Parascaris equorum* revealed the existence of two morphologically indistinguishable varieties, one with a single chromosome pair (univalens variety) and the other with two chromosome pairs (bivalens variety) (*1, 2*). The results of a series of electrophoresis studies have revealed a degree of isolation between the univalens and bivalens varieties, suggesting that they belong to two different diploid species for which the names *P. equorum* (karyotype $2n = 4$) and *P. univalens* (karyotype $2n = 2$) were proposed (*3*). The chromosomes of these two species, which appear similar in germ-cell metaphases when conventional staining techniques are used, are polycentric or holocentric (*4, 5*) and undergo chromatin diminution during development (*1*).

Diminution consists of chromosome fragmentation and the elimination of heterochromatin in presomatic cells. The result is the generation of a difference in chromosome number and quality between germ and somatic cells. Because it has been shown that the maintenance of germ line–limited chromatin is necessary for the germ line quality of blastomeres, diminution seems to be a good example of cell differentiation associated with genomic change (*6, 7*).

Attempts to characterize germ line–limited DNA in various species of *Ascaris* have produced conflicting results. Some studies suggest that this DNA is

composed of repetitive and unique sequences in approximately equal proportions (*8*); others indicate that it is composed exclusively of highly repetitive sequences (*9*). This discrepancy leads to the question of whether there is a difference in chromosome organization between these two species and whether chromatin elimination might involve euchromatin as well as heterochromatin in one or both species.

We now discuss the results of our cytological analysis of the chromosomes in germ and somatic cells of the two *Parascaris* species. Chromosome preparations were treated with Hoechst 33258 stain (*10*) and then a C-banding procedure which permits specific staining of the heterochromatic chromosome regions (*11*). The results showed a difference in chromosome structure between the two species and that the germ line–limited chromatin is overwhelmingly, if not exclusively, heterochromatin.

Parascaris univalens gonial metaphase chromosomes stained with Hoechst 33258 are composed of a weakly fluorescent central segment surrounded by brightly fluorescent blocks (Fig. 1a). The C-banding pattern (Fig. 1b) implies that the central part corresponds to euchromatin and that the surrounding blocks correspond to heterochromatic regions. The banding pattern in corresponding metaphase chromosomes of *P. equorum* reveals blocks of moderately bright fluorescence at both ends and a series of brightly fluorescent regions alternating with weakly fluorescent regions (Fig. 1c). Because both the terminal blocks and the alternating brightly fluorescent regions were positively stained by the C-banding procedure (Fig. 1d), it appears that in these chromosomes there is intercalary heterochromatin. The different fluorescence intensity suggests that in *P. equorum* the terminal heterochromatic regions have different cytochemical characteristics from those in *P. univalens*.

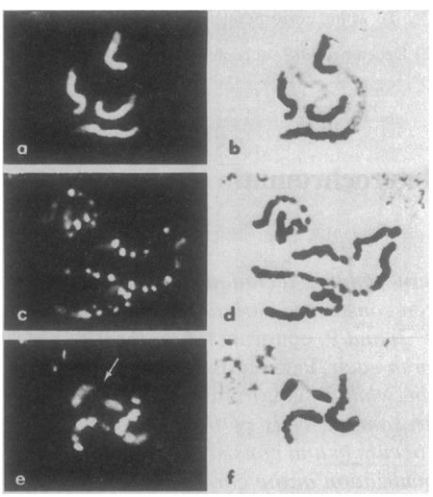

Fig. 1. *Parascaris* gonial metaphases stained with Hoechst 33258 (0.5 μg/ml stain concentration) and sequentially stained by C-banding procedure. (a and b) *Parascaris univalens* metaphase. The chromosomal bright blocks (a) correspond to the positive C-banded regions (b). (c and d) *Parascaris equorum* metaphase. The chromosomes show moderately bright fluorescent blocks at the two ends and a series of brightly fluorescent regions that are intercalated with dull regions (c). Both terminal blocks and brightly fluorescent regions are positive C-banded (d). (e and f) A hybrid between *P. univalens* and *P. equorum* showing the maintenance of the species-specific chromosome banding patterns. The arrow indicates the chromosome from *P. univalens*. The chromosomes from *P. equorum* are two variants that lack the terminal blocks at one end. Chromosome preparations were obtained from the gonadal proximal tract of adult males and females by fixation in a 75 percent methanol:25 percent acetic acid solution and squashing in 40 percent acetic acid.

An analysis of the chromosomes in presumably sterile hybrids between the two species (Fig. 1, e and f) shows unambiguously that the difference between the chromosomes of *P. univalens* and *P. equorum* are species-specific structural qualities. However, *P. equorum* chromosomes are highly polymorphic in the distribution, as well as in the number, of interspersed heterochromatic regions to such an extent that in most cases the homologous pairs could not be recognized. The banding pattern is nonetheless invariant in all the cells of a single individual. Analysis of chromatin diminution confirms the earlier discovery (*1*) that in *P. univalens* only the heterochromatic ends are cast off into the cytoplasm (Fig. 2f). In contrast, Fig. 2, a and b, shows two *P. equorum* cells that have undergone chromatin diminution with a separation of the small, interspersed, brightly fluorescent blocks from the weakly fluorescent blocks. Examination of the anaphase stage (Fig. 2, c and d) in cells of the same kind reveals that the small, weakly fluorescent segments have migrated to the poles while all the bright fluorescent material remains in the equatorial region. Consequently, the *P. equorum* embryo shown in Fig. 2e appears studded with floating fluorescent material that corresponds to the heterochromatin eliminated during earlier cleavage divisions. In this species, therefore, both the terminal heterochromatin and the intercalary heterochromatin are eliminated.

The evolutionary relation between these two *Parascaris* species might involve *P. univalens* giving rise to *P. equorum* by tetraploidization and subsequent inactivation of half the genome by interstitial heterochromatization. Alternatively, one might imagine the derivation of *P. univalens* from *P. equorum* by loss of intercalary heterochromatin and subsequent assembly of the euchromatin in only two chromosomes. This would also explain why, unlike other species of *Ascaris* (*12*), chromatin diminution in *P. univalens* is accompanied by fragmentation of chromosomes; this would be the

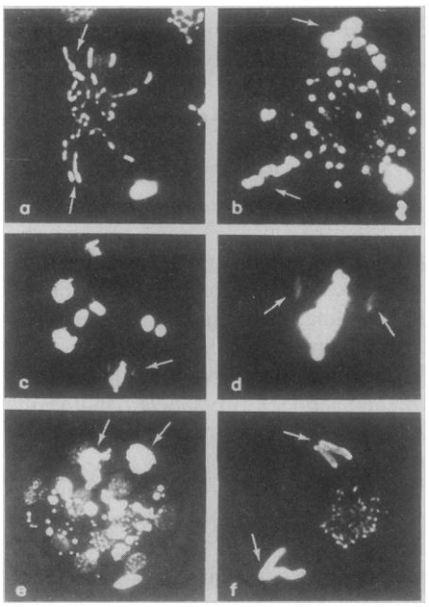

Fig. 2. Chromatin diminution in embryonic somatic cells of *P. equorum* and *P. univalens* stained with Hoechst 33258 (5 μg/ml stain concentration). (a) One *P. equorum* cell is preparing itself for the process of chromatin elimination. Note that with 5 μg/ml stain concentrations, the heterochromatic blocks of *P. univalens* are brightly fluorescent as with 0.5 μg/ml (see Fig. 1a). Unlike what is seen with 0.5 μg/ml (Fig. 1c), the terminal heterochromatic blocks of *P. equorum* at this higher stain concentration also appear bright. Thus at 5 μg/ml concentrations, Hoechst 33258 can identify most, if not all, of the heterochromatic material of both *P. univalens* and *P. equorum*. (b) Another *P. equorum* cell that has undergone the process of chromatin elimination. Note that the euchromatic, dully fluorescent segments in many cases are clearly separated from the intercalated, brightly fluorescent segments. The arrows indicate the terminal heterochromatic blocks. (c) Embryo of *P. equorum* with a cell in anaphase showing chromatin diminution (arrow). (d) The same anaphase at higher magnification; at the two poles there is only dully fluorescent material (arrows), whereas all brightly stained material remains in the equatorial plane. (e) Embryo in a later stage studded with floating fluorescent material that has been eliminated in previous elimination processes. The arrows indicate two germ cells which have retained the heterochromatin. (f) A cell that has undergone the process of chromatin diminution in a *P. univalens* embryo. The terminal fluorescent blocks are cast-off (arrows). Note the similarity between the euchromatic segments in this metaphase and the dully fluorescent segments in the metaphase shown in (b). Embryonic cells were observed in ova taken from uteri and grown at 37°C for various lengths of time. Fixation was in a 75 percent methanol:25 percent acetic acid solution.

remains of a mechanism that had been necessary for the elimination of intercalary heterochromatin. In either case, the cytological data support the electrophoresis data, which show that, although there is an almost identical morphology, the separation of *P. univalens* from *P. equorum* is ancient (about 10 million years) (*3*). A similar situation has been found in the twin species of nematodes *Caenorhabditis elegans* and *C. briggsae*, which by nucleotide sequence divergence appear to have separated 10 million years ago although they are almost identical morphologically (*13*).

With respect to the DNA sequences eliminated in somatic cells during early embryogenesis, we have determined that the amount of heterochromatin in *P. univalens* metaphases is about 70 percent of the total length of all the chromosomes. The amount of germ line–limited satellite DNA is about 85 percent of the total DNA (*9*). If one assumes a similar DNA content per unit chromosome length in euchromatin and heterochromatin, the near equivalence between these two measures supports the view that in these species the germ line–limited DNA is composed almost exclusively of highly repetitive DNA (*9*).

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Phenotypic Variation Within Histocompatibility-Defined Clones of Marine Sponges

Abstract. *Nongenetic phenotypic variation can be identified by its occurrence within genetically uniform clones. A histocompatibility bioassay of clonal identity was used to ascertain the extent of phenotypic variation within natural clones of two species of marine sponges. Multiple morphological forms of the sponge* Aplysina fistularis *were found to occur within single clones, indicating a nongenetic polymorphism. In contrast, a genetic basis is suggested for a polymorphism of* Aplysina cauliformis; *within single clones of this species, individuals were uniform in color and morphology.*

A necessary concern of evolutionary biology is the distinction between genetic and nongenetic components of phenotypic variation in nature. Conventional heritability studies are limited to those organisms that can be bred under controlled conditions. Ideally, sets of genetically equivalent individuals should be examined to isolate environmental and developmental influences on phenotypic expression. Among plants and lower invertebrates, replicates of single genotypes in natural populations are produced by clonal propagation. If such clones can be identified, the natural range of phenotypic expression displayed by single genotypes can be observed. Self-recognition phenomena have potential use for the identification of clonal lineages. The capacity for self versus nonself discrimination in intraspecific contacts has been demonstrated for various lower invertebrates, including sponges and cnidarians. Distinct behavioral responses (*1*) and histocompatibility-like responses (*2*) distinguish allogeneic from isogeneic contacts between individuals. Where asexual modes of reproduction have structured populations of sessile marine invertebrates into clonal lineages, it has been possible to utilize these phenomena as bioassays of clonal identity. Self-recognition bioassays have been used to assess the spatial distributions of clones which were then analyzed with respect to demographic and ecological factors for sponges (*3, 4*), reef-building corals (*5*), and sea anemones (*6*). We report here an extension of this approach to examine variation in phenotypic expression for individual clones of two species of marine sponge.

Aplysina fistularis (*Verongia fistularis*) and *Aplysina cauliformis* (*Verongia longissima*) are common Caribbean reef sponges occurring at depths from 5 to 30 m (*7*). The typical growth form of *A. fistularis* is a hollow cylinder, occurring either individually or in clusters up to a meter in height and 10 cm in diameter. This form has been designated *A. fistularis fistularis* by van Soest (*8*). Other morphotypes have been characterized, in part, by the presence of structures that either extend from the apices of the tubes as slender digitate processes (less than 2 mm in diameter) or arise from a massive or encrusting base as erect branches (up to 3 cm in diameter). These forms have been designated as *A. fistularis insularis* and *A. fistularis fulva*, respectively (*8*). *Aplysina cauliformis* is a ropelike or branching sponge, with a typical diameter of 1 to 2 cm and ranging in color from dark blue to yellow (*7*).

Grafting bioassays were used (i) to assess the accuracy of the bioassays in distinguishing clones and (ii) to determine the range of phenotypic expression for selected traits within single clones. Field work was undertaken at the Discovery Bay Marine Laboratory, in Jamaica, and at the NOAA Hydrolab facility in St. Croix, U.S. Virgin Islands.

Grafting experiments were performed on sponges that were selected from relatively large areas so that many different clones would be included. A total of 80 *A. fistularis* grafts were performed in situ on Arena Reef, in Discovery Bay, over an area of approximately 1000 m^2. A disk of tissue was removed from a donor sponge with a cork borer (1/2-inch diameter) and inserted into a hole in the recipient sponge made in the same manner. For *A. cauliformis*, 188 grafts were made on the outer eastern slope of Salt River Canyon, St. Croix, in an area of approximately 600 m^2. A segment of branch 2 to 5 cm in length was cut from a donor sponge and tied to a branch on a recipient sponge with nylon monofilament. For both species, a numbered identification tag was attached to each graft. Within 3 to 5 days, two distinct graft responses could be observed. A graft acceptance was characterized by the fusion of donor and recipient tissues. No tissue fusion occurred in rejection responses; instead a pronounced cuticle developed on the interfacing surfaces of the donor and recipient tissues. Scoring of grafts was coded "blind" with respect to expected outcomes.

Experimental controls indicated accurate identification of clones by the histocompatibility bioassay. (i) All autografts between parts of the same sponge, 9 for