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Electrophoretic Evidence for Self-fertilization in Two Species of Spirorbid Polychaetes

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Abstract.—Electrophoretic evidence for self-fertilization in two species of spirorbid polychaetes by Richard Beckwitt, *Bull. Southern California Acad. Sci.*, 81(2):61–68, 1982. The inheritance of electrophoretically detectable enzymatic variation was studied by means of laboratory culture of *Pileolaria pseudomilitaris* and *Janua brasiliensis*. These variants are inherited as simple Mendelian codominants in single-pair matings. A small percentage of progeny from single-pair matings of each species are produced by self-fertilization in these hermaphroditic worms. Isolated individuals of *P. pseudomilitaris* produced reduced numbers of progeny with genotypes that confirmed self-fertilization by the parent. Isolated individuals of *J. brasiliensis* did not reproduce in culture. The high degree of polymorphism at several loci suggests that self-fertilization is not a common occurrence in natural populations.

Introduction

Worms of the polychaete family Spirorbidae are small, sessile filter-feeders. They are often locally abundant, especially on harbor floats and ship hulls. Spirorbids are short-lived and easily cultured in the laboratory. All species of Spirorbidae that have been examined have proven to be hermaphroditic, with functional eggs and sperm present simultaneously (Gee and Williams 1965). Those species investigated have some capacity for self-fertilization (Potswald 1968). Fertilization is presumed to occur either as the eggs are being deposited or shortly after. Daly and Golding (1977) have demonstrated a sperm storage organ at the base of the tentacular crown. Sperm, released into the water, are apparently collected by the feeding apparatus of other individuals and stored.

Several species of spirorbids are commonly found in southern California. Among these, two of the most abundant are *Pileolaria pseudomilitaris* (Thiriot-Quievreux), and *Janua brasiliensis* (Grube). The latter species has been recently redescribed by Knight-Jones, Knight-Jones and Kawahara (1975). Both species are found together attached to *Mytilus edulis* shells on floating docks. In addition, *P. pseudomilitaris* is found attached to rocks in mid-intertidal pools.

Many aspects of reproductive biology, including self-fertilization, can be studied directly if there are genetic markers that allow the genotype of each individual to be determined. Enzyme electrophoresis provides a useful tool in such investigations, since several independent, polymorphic loci can be scored in a single individual. I have examined the inheritance of electrophoretic phenotypes (allozymes) in spirorbids reared in the laboratory. In this paper, I present data on the Mendelian inheritance of allozymes at seven loci, and also evidence for a small amount of self-fertilization in *Pileolaria pseudomilitaris*. Similar data are presented for three loci of *Janua brasiliensis*.

Materials and Methods

Laboratory Culture

Spirorbids were cultured using methods developed by Shisko (1975). Cultures were maintained at 16–18°C in aerated, non-circulating aquaria. Sea water was obtained from Marineland of the Pacific (this water was filtered through a coarse gravel filter). All cultures were started with newly settled juveniles. Larvae were allowed to settle in a covered plastic Petri dish (50 mm diameter), in about 5 ml of sea water. After settlement, the bottom half of the Petri dish was transferred to a one liter aquarium. Cultures were fed a suspension of the single-celled green alga *Dunaliella tertiolecta* and some finely ground Tetra-Min (Tetra KraftWerke, W. Germany), and the water changed every two weeks.

Electrophoresis

Horizontal slab starch gel electrophoresis was used exclusively. Individual worms were removed alive from their tubes, using fine jeweler's forceps and #0 insect pins. Each worm was placed in a small mortar and pestle and ground in 5 μl or less of deionized water with the aid of less than 1 mg of fine carborundum powder. Six enzyme stains were used: aspartate amino transferase (AAT, EC 2.6.1.1), a non-specific esterase (EST), glucosephosphate isomerase (GPI, EC 5.3.1.9), leucine amino peptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucomutase (PGM, EC 2.7.5.1). Stain recipes were from Selander, Smith, Yang, Johnson and Gentry (1971). The stain and buffer combinations used are described in Beckwitt (1980).

Data were recorded for each band as distance traveled from the origin, divided by the distance traveled by the tracking dye, bromphenol blue, multiplied by 100. This relative mobility value allowed comparisons from one gel to another, even though the small amount of sample made it impossible to run any individual more than once. Alleles were named in terms of this mobility value (e.g., pgi^{20} is the allele of the PGI locus with a relative mobility of 20 units compared to bromphenol blue). Although no direct comparisons were possible, indirect comparisons using albumin and hemoglobin standards, as well as comparisons among individuals with a restricted number of known genotypes, and enzymes with little or no variability, showed relative mobility values to be similar from one gel to the next (± 2 units). This degree of error was less than the usual difference between two alleles (it was about the same as the thickness of a single band). In cases where suspected alleles differed by distances of the same magnitude, they were treated as being the same.

Genetic Analysis

The genotypes of parents were compared with the genotypes of their F_1 offspring. Results were interpreted on the basis of a simple Mendelian model of multiple codominant alleles at a single locus. One or two larvae were allowed to settle in Petri dishes. These worms were reared in one liter aquaria as described above until they had produced one or more broods of offspring. The parents were then removed, and their genotypes determined for three enzymes. When a majority of the offspring had matured (determined by the production of F_2 offspring) they too were examined for genotypes at the same loci as their parents.

Sampling

Specimens of *Janua brasiliensis* used to initiate cultures were taken from *Mytilus edulis* shells attached to floating docks at Holiday Harbor Marina, San Pedro Harbor, Los Angeles County. Some specimens of *Pileolaria pseudomilitaris* used to initiate cultures were taken from Holiday Harbor Marina, others were taken from stones in mid-intertidal pools at Royal Palms Beach, Palos Verdes Peninsula, Los Angeles County.

Results

The results of all crosses between pairs of larvae of *Pileolaria pseudomilitaris* are given in Table 1. Cultures 1 through 11 in this table were started with a pair of larvae from Holiday Harbor (floating docks). Five additional cultures, 12 through 16, were started with one larva from Holiday Harbor and one from Royal Palms (intertidal habitat). For each culture, variation was scored for three enzymes: GPI, EST, and LAP or PGM, MDH, and AAT. In each case, the F_1 offspring are only those expected from the parental genotypes. Note that there is no evidence for reproductive isolation between the two populations from different habitat types.

Similar results were obtained for *Janua brasiliensis* (Table 2). In this case, two cultures were stained for the GPI locus and three for the PGM and MDH loci. Successful stain and buffer combinations for EST, LAP, and AAT were not discovered for this species.

In *Pileolaria pseudomilitaris,* there are multiple loci controlling PGM and EST expression. Large numbers of bands, of unequal intensity, are often closely spaced on a gel. Through formal genetic analysis, it was possible to assign bands to separate loci. For each enzyme system, one locus consistently stained more darkly. Only variation at this locus was scored. In addition, there are two loci with AAT activity that probably correspond to the mitochondrial and cytosolic forms in other animals. Both loci stain darkly and are well separated on a gel, so both loci were scored.

Variation at the MDH locus is rare for both species, as it is for AAT-1 and AAT-2 of *P. pseudomilitaris.* None of the rare alleles appeared in any culture tested. The allelic nature of variation at these loci can only be inferred from negative evidence: no alternate alleles appeared among progeny when they were absent from the parents.

Evidence for Self-fertilization

As Tables 1 and 2 show, certain offspring in some cultures of both *Pileolaria pseudomilitaris* and *Janua brasiliensis* could only have been produced by self-fertilization. There is evidence for self-fertilization in 8 of 16 *P. pseudomilitaris* cultures and in 1 of 5 *J. brasiliensis* cultures. For *P. pseudomilitaris,* GPI and EST were scored for the same individuals. Data from these two independent loci were consistent in indicating which progeny were produced by self-fertilization.

Some cultures of each species were attempted with single, newly settled larvae reared in isolation. Of these, no *J. brasiliensis* produced any offspring. Instead, worms produced three or more successive batches of embryos that were held in the brood chamber for up to one month, but never developed past the 32–64 cell stage. In one case, when a second immature worm was added to a Petri dish in

Table 1. Inheritance of allozymic variation: *Pileolaria pseudomilitaris*. F_1 genotypes given are only those found in that culture. Alleles are named in terms of their mobility relative to bromphenol blue. F_1 genotypes marked with an asterisk (*) are the product of self-fertilization; those marked with a dagger (†) are the product of cross-fertilization; the rest are unresolved.

Locus	Culture	P_1 Genotypes	F_1 Genotypes	Number
GPI	1	1/1 × 5/5	1/5	13†
	2	1/1 × 1/5	1/5	16
			5/5	4*
	3	1/1 × 1/5	1/1	10
			1/5	5
			5/5	4*
	4	1/1 × 1/5	1/1	12
			1/5	9
	5	−5/1 × 1/10	−5/1	3
			1/10	5
	12	1/1 × 20/20	1/20	9†
			20/20	4*
	13	−5/1 × 5/20	−5/5	5†
			−5/20	2†
			1/5	2†
			1/20	4†
			5/5	1*
			20/20	1*
	14	1/5 × 1/20	1/1	4
			1/20	4
			5/20	3†
	15	1/10 × 20/20	1/20	7†
			10/10	1*
			10/20	2†
	16	5/5 × 1/20	1/5	6†
			5/20	10†
PGM	6	50/50 × 46/54	46/50	9†
			50/50	1*
			50/54	8†
	7	46/50 × 46/50	46/46	10
			46/50	6
			50/50	3
	8	50/50 × 46/54	46/50	2†
			46/54	5*
			50/50	1*
			50/54	11†
			54/54	1*
	9	46/50 × 50/54	46/50	6
			46/54	4†
			50/50	8
			50/54	3
	10	46/46 × 46/54	46/46	22
			46/54	12
			54/54	4*
	11	40/40 × 40/40	40/40	14

Table 1. Continued.

Locus	Culture	P_1 Genotypes	F_1 Genotypes	Number
EST	1	52/56 × 52/56	52/52	4
			52/56	8
			56/56	2
	2	56/66 × 60/60	56/56	2*
			56/60	3†
			56/66	3*
			60/60	1*
			60/66	2†
			66/66	5*
	5	60/66 × 66/66	60/66	3
	12	56/60 × 56/60	56/56	3
			56/60	7
			60/60	3
	13	60/60 × 56/60	56/60	9
			60/60	6
	15	56/60 × 60/66	56/60	1
			56/60	3†
			60/60	2
			60/66	3
	16	60/60 × 60/66	60/60	9
			60/66	7
LAP	5	30/30 × 30/36	30/36	8
	12	30/30 × 30/36	30/30	3
			30/36	9
			36/36	1*
	13	27/27 × 30/30	27/30	13†
			30/30	2*
	16	30/30 × 36/40	30/36	8†
			30/40	8†

which the single individual had produced four aborted broods, both individuals subsequently produced normal broods that matured and reproduced as well.

Of eight attempted cultures of *P. pseudomilitaris* started with single individuals, three produced F_1 offspring; the others produced one to three aborted broods. None of the progeny produced by self-fertilization reached sexual maturity. In one case, a few immature individuals were used for electrophoresis. The parent was heterozygous at the GPI locus and offspring included both heterozygotes and homozygotes for the parental allozymes.

Fertilization in the field appears to be from at least a few neighboring individuals. Adults with broods of embryos ready to hatch were collected from the field, the offspring reared to maturity, and their genotypes compared to that of the parent. In all cases, all the offspring shared at least one allele with the parent. In one brood, four other alleles were also present at the GPI locus of the offspring, indicating fertilization by sperm from at least two other adults. At least one or two alleles not present in the known parent were always found in the offspring, indicating that self-fertilization does not play a major role in natural populations.

Table 2. Inheritance of allozymic variation: *Janua brasiliensis.*

Locus	Culture	P_1 Genotypes	F_1 Genotypes	Number
GPI	1	−4/1 × 1/1	−4/1	8
			1/1	11
	2	1/1 × 1/6	1/1	15
			1/6	5
PGM	3	36/42 × 36/42	36/36	4
			36/42	16
	4	32/36 × 32/42	32/32	3
			32/36	4
			32/42	14
			36/36	5*
			36/42	11†
	5	42/42 × 42/42	42/42	15

Discussion

Surveys of allozymic variation are becoming increasingly common in the literature of population genetics. Progeny testing is not often done prior to such surveys. Sometimes this is due to the technical difficulties of breeding a given species. Often, progeny testing is not considered necessary since the pattern of bands on electrophoretic gels appears to conform to a Mendelian interpretation. It is often assumed that different mobility classes of an enzyme can be translated directly into genotypes at the locus encoding for that enzyme. However, there are difficulties with this assumption. Johnson (1973) has discussed some of the problems that can confound interpretation of electrophoretic data. I have found the interpretation of data, especially from field samples, to be simplified greatly because of the results of progeny testing. In this way, the presence of two or more loci encoding for enzymes with similar activities can be detected, and the alleles more easily assigned to the correct locus. Proper interpretation of the results for the PGM and EST stains would not have been possible otherwise. Difficulties in the genetic interpretation of variation have been noted in studies of the eel *Anguilla rostrata* (Koehn 1972). Oxford (1975) showed that changes in diet induced phenocopies at an esterase locus in the snail *Cepaea nemoralis.* McGovern and Tracy (1981) were able to change the transferin and LAP "genotypes" of individual voles (*Microtus ochrogaster*) by holding them at different temperatures in the laboratory. Although such findings do not necessarily cast doubt on electrophoretic surveys done without the confirmatory evidence of progeny testing, they do suggest that progeny testing is highly desirable when possible.

Significance of Self-fertilization

A small but detectable amount of self-fertilization is present in laboratory cultures of both *Pileolaria pseudomilitaris* and *Janua brasiliensis.* This supports the earlier results of Gee and Williams (1965) and Potswald (1968) for some other species in the family. Neither previous study was entirely satisfactory. Gee and Williams (1965) used adult animals, collected from the field at a season when the

animals were "entirely lacking in mature eggs and sperm." They were unaware of the presence of a sperm storage organ subsequently described by Daly and Golding (1977). Potswald (1968) used similar techniques, although he did rear a few isolated larvae to maturity and reproduction. In both studies, the progeny of self-fertilization were not held beyond metamorphosis and there was no indication that they were capable of reproduction. Although both studies adequately controlled for the presence of sperm from an outside source, there was no way for the investigators to prove that offspring were from self-fertilization, rather than parthenogenesis or some other process. I found that only a few *Pileolaria pseudomilitaris* reproduced successfully when reared entirely in isolation, and their progeny did not survive or grow well. Conclusive evidence that these progeny were the product of self-fertilization was provided by allozyme markers. In similar cultures of *Janua brasiliensis* survival and growth were even less.

In cultures initiated with pairs of worms, evidence from allozyme markers indicated that both species produced a small number of progeny by self-fertilization. Results of two cultures of *P. pseudomilitaris* are particularly informative: PGM culture 8 and EST cultures 2 (see Table 1). In each of these two cases, one parent is a heterozygote for two alleles that are not found in the other parent. Some individuals of the same heterozygous genotype are found among the F_1 offspring. These offspring can not be produced by any simple mechanisms of parthenogenesis except perhaps from diploid oocyctes. If parthenogensis were by diploid eggs, it is difficult to explain the presence of some homozygotes in the same cultures. A minimum estimate of the proportion of self-fertilization in culture can be made by summing all of the progeny known to be from self-fertilization and dividing by the total number of progeny from all cultures. This gives an estimate of 9.8% (22/276) of *P. pseudomilitaris* and 4.5% (5/111) for *J. brasiliensis* (based on data from the GPI and PGM loci). It is not possible to estimate the amount of self-fertilization that occurs in natural populations. Since natural populations are usually dense and water currents can easily carry sperm between neighbors, it is likely to be less than that in the laboratory.

Self-fertilization probably plays only a minor role in well-established *P. pseudomilitaris* populations. One brood from a single naturally fertilized parent often has three or more alleles at several loci. It is feasible that a single individual could found a new population, producing the first generation entirely by self-fertilization. Such an event can not be common since most natural populations are highly polymorphic, often with four or five alleles at each of several loci (Beckwitt 1980). As an event of evolutionary consequence, however, such a rare occurrence could be highly significant. It provides a way for spirorbids to colonize new habitats and may account in part for the degree of ecological speciation seen in the family.

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