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* Present address: Medical Research Center, Prince Henry's Hospital, Melbourne, Australia.

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Sibling Species in the Marine Pollution Indicator *Capitella* (Polychaeta)

Abstract. *Electrophoretic patterns for eight enzyme loci clearly distinguish six sibling species in the well-known pollution indicator worm,* Capitella capitata. *Unlike sibling species of* Drosophila, *the* Capitella *species have virtually no alleles in common. Close examination has revealed only slight morphological differences between species, while life histories and reproductive modes are very distinct. The* Capitella *species are ideally suited for genetic and evolutionary studies.*

The polychaete worm *Capitella capitata* has been regarded as an excellent indicator of pollution or environmental disturbance (*1*). This so-called cosmopolitan species has been found in every part of the world where marine benthic studies have been conducted. Our results show that it is, in fact, a complex of as many as six sibling species.

We have examined life histories, reproductive modes, and morphologies of this worm, and the electrophoretic patterns for the following enzymes: phosphohexose isomerase (PHI), xanthine dehydrogenase (XDH), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH-1 and IDH-2), malate dehydrogenase (MDH-1 and MDH-2), and α-glycerophosphate dehydrogenase (α-GPDH) (*2*). Samples of five *Capitella* populations in the vicinity of Woods Hole, Massachusetts, and two populations from Gloucester, Massachusetts, collected at intervals for more than a year, consist of six sibling species. Although allelic variation occurs within each species, we have not detected any gene exchange between species. Table 1 lists morphological and life history features of the six species. It is apparent that the morphological differences are slight and that there is some overlap in life histories. However, considering the eight genetic loci listed above, only two alleles are found in common between any pair of species—the monomorphic PGM in species I and Ia, and the monomorphic MDH-1 in species II and IIa (*3*). More limited results on two additional loci (a single esterase and a single leucine aminopeptidase locus) also show no alleles in common between the species. In Table 1 the species have been numbered according to the relative electrophoretic mobilities of their most common PHI alleles, with bovine serum albumin (1.0) as a standard: species I (0.48); Ia (0.50); II (0.29); IIa (0.36); III (0.18); and IIIa (0.16). In Table 2 the mobilities of all PHI alleles relative to species I PHI (the most common allele) in a tris-maleate buffer system are given. There are no alleles in common between any two species.

The individuals of all six species have been maintained in the laboratory in standing seawater at constant temperature, and azoic mud was provided as food. Species I and II are ideal laboratory animals in that they have a short generation time (30 to 40 days at 20°C, depending on the abundance of food); we have now carried them through many generations. The genetic systems at the eight loci have been confirmed for these two species by comparing the electrophoretic mobilities of enzymes in parents and offspring from a number of crosses. All the differences between species were consistent, whether the individuals were brought directly from the field or reared under constant laboratory conditions for several generations. We have not found any evidence for environmen-

Table 1. Features of morphology and life history in six sibling species of *Capitella*. There are three thoracic setigers with capillary setae in species III [compare, *Capitellides jonesi* Hartman 1959 (*14*)], and seven in the other species. In the formulas for teeth above the main fang of the hooded, abdominal hooked setae, the first row of teeth is immediately above the fang. Abbreviations: P, protandrous; ♂, male; ♀, female; ☿, hermaphrodite.

Species	Head		Tail		Teeth above fang		Weight of worms* (mg)	Sex	Eggs (*6*)		Larvae in plankton
	Prostomium	Peristomium	Shape	Dorsal cleft	Row	Formula			Diameter (μm)	No./brood	
I	Broad, triangular	Short	Plain	Absent	3 2 1	3 to 5 5 5	3 to 12	♂,♀,P☿†	260 by 180	30 to 400	Several hours
Ia	Sharply conical	Partly fused	Plain	Absent	3 2 1	0 to 5 4 to 6 5	10.1	♂,♀,?☿	75	200 to 2000	Several days
II	Long, triangular	Partly fused	Lobed	Present	3 2 1	0 to 3 3 to 4 4	12.0	♂,♀,P☿	230	30 to 400	6 to 24 hours
IIa (*15*)	Like II	Like II	Plain	Absent			11.9				
III	Broad, triangular	Distinct, very long	Flared	Present	3 2 1	3 to 4 4 to 5 4 to 5	1 to 4	P☿	50	200 to 1000	≤ 2 weeks
IIIa	Broad, triangular	Partly fused	Lobed	Present			1.7	♂,♀	250	30 to 50	None

*Average wet weight. †Occasional self-fertilization.

tally induced electrophoretic variation in the thousands of animals that have been studied. Species III (the obligate outcrossing hermaphrodite) does mate and reproduce in the laboratory, but we have so far been unsuccessful in inducing the small, long-lived planktonic larvae to metamorphose. Species Ia has a longer generation time than species I and II, and we know relatively little about the life histories of species Ia, IIa, and IIIa. Figure 1 indicates some of the differences in size and degree of development of the larvae of the different species as they hatched spontaneously from egg cases kept in the laboratory (also Table 1). Before all the electrophoretic results were available we made extensive attempts to crossbreed pairs of some of the species without success. More than 200 attempts to cross pairs of males and females from species I with Ia, II, and III, and from species II with III, never resulted in any egg cases. Pairs made within species I, II, or III invariably produced egg cases. The lack of success in crosses between species is not surprising considering the almost total lack of alleles in common.

Dramatic differences occur in the percent occurrence of the six *Capitella* species in samples collected at seven stations in Massachusetts at different times of the year. Species I is the most common species in intertidal samples in Buzzards Bay, except at some stations during the winter months December–March, when species III may predominate during its breeding season. Species Ia also occurs in large numbers only for a limited part of the year during the spring and early summer. Two grab samples (1/25 m^2 each) taken at the Woods Hole sewer outfall in January and September both had five species of *Capitella* but differed radically in the relative species abundance. Subtidal samples taken less than a mile apart at Gloucester on the same date differed markedly in their species composition. In one, species Ia formed more than 90 percent of the *Capitella* populations; in the other, species IIIa formed more than 90 percent of the sample.

Table 2. Mobility of PHI alleles in six species of *Capitella*. Two X's indicate the most common allele in a given species (*15*).

M*	Species					
	Ia	I	IIa	II	III	IIIa
1.06	XX					
1.00		XX				
.87	X					
.84		X				
.83			XX			
.68				XX		
.34					XX	
.30						XX
.20					X	
.13						X

*Mobility of PHI alleles relative to most common PHI allele in species I (tris-maleate buffer).

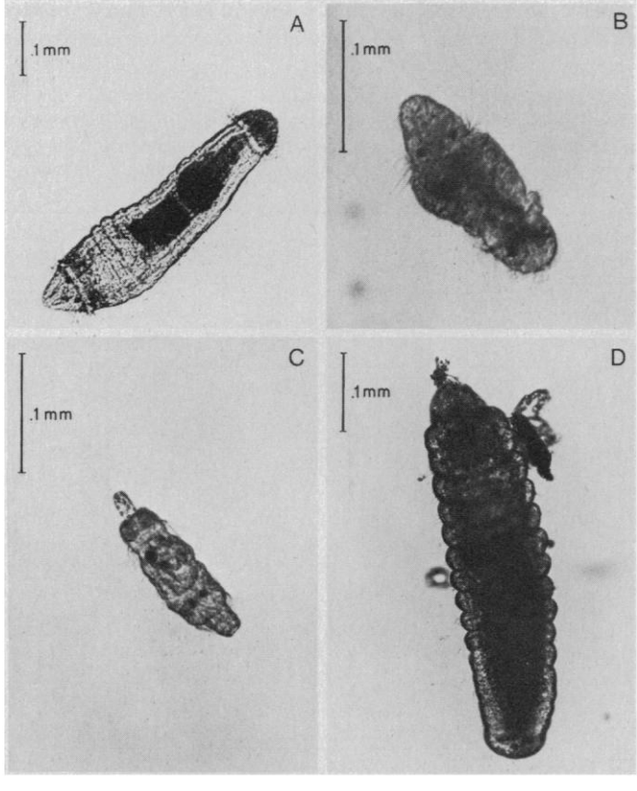

Fig. 1. Newly hatched larvae of *Capitella* species. (A) Species I, (B) species II, (C) species III, and (D) species IIIa.

Our discovery that as many as five species of *Capitella* occur in a single sample is not unique. Hartman (*4*) found four closely related species of *Capitella* in samples taken in areas of waste disposal in San Pedro and Santa Monica bays, but did not describe or name the species. The problem of determining which of our six species have previously been described is a difficult one, complicated by the fact that many of the previous descriptions are inadequate. It will be necessary to collect in many of the type localities and to make parallel studies of morphology, life histories, and genetic variation. As collections are extended over a wider geographic area other distinct sibling species will be discovered.

The six species of *Capitella* represent distinct temporal adaptations to disturbed environments, based partly on their dispersal capabilities, and partly on the relative length of their breeding seasons (*5*). Adaptations to differing patterns of temporal variation are well documented in *Drosophila* (*6*), and *Gerris*, a genus of water striders (*7*). There may be many examples of groups of closely related, relatively opportunistic species, that may be distinguished by their life histories. Some of the best known marine examples belong to the following genera: *Crepidula* (*8*), *Littorina* (*9*), *Jaera* (*10*), *Tisbe* (*11*), and *Ophryotrocha* (*12*). Others yet to be described in detail are suggested by reports of two kinds of larvae occurring within a single species (*13*).

The lack of allelic similarity suggests that chromosomal events have been involved in speciation. The very different life histories and short generation times in a group of sibling species with similar habitat and food requirements, make *Capitella* ideal material for comparative studies of adaptation and genetics. The use of *Capitella capitata* as a pollution indicator will have to be modified—the large differences in life history suggest that each species may provide even more sensitive indications of previous patterns of disturbance.

JUDITH P. GRASSLE
Marine Biological Laboratory, Woods Hole, Massachusetts 02543
J. FREDERICK GRASSLE
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

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2. Electrophoretic results were obtained by vertical starch-gel electrophoresis in 12 to 13 percent Connaught hydrolyzed starch; the following electrode and gel buffers were used. For PHI and XDH, the electrode buffer consisted of 0.5*M* tris, 0.5*M* H_3BO_3, 0.016*M* EDTA, *p*H 8.0; and the gel buffer consisted of a 1 : 4 dilution of the electrode buffer with distilled water. For PGM and IDH, the electrode buffer consisted of 0.1*M* tris, 0.1*M* maleic anhydride, 0.0084*M* EDTA, 0.01*M* $MgCl_2 \cdot 6H_2O$, and sufficient NaOH (~ 0.124*M*) to bring the *p*H to 7.45; and the gel buffer consisted of a 1 : 9 dilution of the electrode buffer with distilled water. This buffer system was also used for PHI, and gave better resolution of species I and Ia PHI, and species III and IIIa PHI. For MDH and α-GPDH, the electrode buffer was 0.223*M* tris, 0.086*M* citric acid, and sufficient NaOH (~ 0.028*M*) to bring the *p*H to 6.45; and the gel buffer was 0.016*M* tris, 0.0104*M* citric acid, and sufficient NaOH (~ 0.002*M*) to bring the *p*H to 6.85. Individual worms with their guts cleared were homogenized in an extractant containing 0.01*M* tris, 0.0072*M* HCl, 0.001*M* EDTA, $5 \times 10^{-5}M$ NADP, 0.25*M* sucrose, *p*H 7.3. Staining mixtures were as follows: for PHI, 100 ml of 0.05*M* tris-HCl buffer, *p*H 8.0, 4.0 ml of 0.12*M* $MgCl_2 \cdot 6H_2O$, 40 mg of MTT tetrazolium, 100 mg of D-fructose 6-phosphate, 30 mg of NADP, 10 mg of phenazine methosulfate (PMS), and 50 units of glucose-6-phosphate dehydrogenase (Sigma type XI); for XDH, 100 ml of 0.1*M* tris-HCl, *p*H 8.0, 2 ml of 0.12*M* $MgCl_2 \cdot 6H_2O$, 100 mg of hypoxanthine, 30 mg of nitro blue tetrazolium, 10 mg of MTT tetrazolium, 50 mg of NAD, 5 mg of PMS; PGM, 100 ml of 0.05*M* tris-HCl, *p*H 8.0, 5 ml of 0.12*M* $MgCl_2 \cdot 6H_2O$, 150 mg of α-D-glucose 1-phosphate, 10 mg of MTT tetrazolium, 10 mg of NADP, 4 mg of PMS, 50 units of glucose-6-phosphate dehydrogenase (Sigma type XI); IDH, 100 ml of 0.1*M* tris-HCl, *p*H 7.0, 1 ml of 0.12*M* $MgCl_2 \cdot 6H_2O$, 125 mg of trisodium isocitrate, 30 mg of nitro blue tetrazolium, 30 mg of NADP, and 2 mg of PMS; MDH, 4.02 g of L-malic acid, 0.758 g of tris, 162 ml of distilled water, sufficient 4.0*M* NaOH to bring the *p*H to 7.05 (~ 14.0 ml), 48 mg of KCN, 75 mg of NAD, 50 mg of tetra nitro blue tetrazolium, and 5 mg of PMS; for α-GPDH, 100 ml of 0.05*M* tris-HCl buffer, *p*H 8.5, 180 mg of EDTA, 20 mg of nitro blue tetrazolium, 30 mg of NAD, 800 mg of DL-α-glycerophosphate (disodium salt), and 5 mg of PMS; the PMS is added after the gel has been staining for 90 minutes in the dark.

3. The mobilities of the most common PHI alleles (relative to 1.0 for PHI species I fast homozygote), and the numbers of alleles (parentheses) are: species I, 1.0 (2); species Ia, 1.04 (2); species II, 0.60 (1); species IIa, 0.77 (1); species III, 0.36 (2); species IIIa, 0.34 (2). The range of mobilities for the XDH alleles for each species, and the number of alleles (parentheses) are: species I, 1.05 to 1.31 (11); species Ia, 0.87 to 1.07 (6); species II, 0.89 to 1.14 (8); species IIa, 0.54 to 0.61 (4); species III, 1.33 to 1.60 (9); species IIIa, 0.97 to 1.13 (7). It is apparent that the XDH alleles for species I, species IIa, and species III are unique, while the mobilities of the XDH alleles in species Ia, II, and IIIa show considerable overlap. However, within each species XDH heterozygotes are only found between alleles with similar mobilities. If XDH alleles do not assort independently within each species as we have defined them, then the identity of XDH with similar mobilities between species seems doubtful. Species III shows a complete lack of PGM and IDH activity (at both loci). The mobilities of the most common PGM alleles (relative to PGM species I fast band) and the number of alleles (parentheses) in the other five species are: species I, 1.0 (1); species Ia, 1.0 (1); species II, 0.57 (1); species IIa, 0.67 (1); species IIIa, 0.50 (3). The mobilities of the most common IDH-1 alleles (relative to PGM I fast band) and the number of alleles are: species I, 1.14 (1); species Ia, 1.07 (2); species II, 1.09 (2); species IIa, 1.13 (1); species IIIa, 0.90 (1). The mobilities of the most common IDH-2 alleles (relative to PGM I fast band) and the number of alleles are: species I, 0.96 (3); species II, 0.79 (2); species IIa, 1.01 (1); species IIIa, 0.44 (2). The mobilities of the most common MDH-1 alleles (relative to MDH-1 species I) and the number of alleles are: species I, 1.0 (1); species Ia, 1.01 (1); species II, 1.02 (1); species IIa, 1.02 (2); species III, 1.04 (1); species IIIa, 0.71 (1). The mobilities of the most common MDH-2 alleles (relative to MDH-1 species I) and the number of alleles are: species I, 0.72 (2); species Ia, 0.71 (2); species II, 0.59 (2); species IIa, 0.61 (2); species III, 0.59 (2); species IIIa, 0.29 (2). The mobilities of the most common α-GPDH alleles are: species I, 1.12 (1); species Ia, 1.09 (1); species II, 1.06 (1); species IIa, 1.02 (1); species III, 0.67 (1); species IIIa, 0.88 (1). These mobilities are repeatable and consistent and have been determined for at least several hundred individuals, except as noted for species IIa in (*15*).

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15. The data on species IIa rests on only three mature individuals, and is therefore incomplete. Several hundred to several thousand individuals in each of the other five species have been examined and subjected to electrophoresis.
16. The egg number per brood is highly dependent on the amount of food available. For each species, the low end of the quoted range represents counts from laboratory individuals in cultures where food was not abundant. The high end of the range for each species is representative of field-caught animals.

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Models of Carcinogenesis as an Escape from Mitotic Inhibitors

Abstract. *Diffusible mitotic inhibitors are assumed to govern proliferation of normal cells. Cancer cells may escape regulation by failing to either recognize or secrete inhibitors. In the latter case, probabilities and expected times for reaching a critical clone size are given. Patterns of proliferation will depend on whether the inhibitor concentration is locally or systemically determined.*

In an adult animal, many tissues, such as skin, liver, or bone marrow, can respond to unusual loss of cells by increased cellular proliferation, which restores the tissue to normal size. Mechanisms that inhibit such proliferation in normal tissue, yet permit it in wounded tissue, are not well understood, but may include mediation by mitotic inhibitors (*1–3*) which are secreted by cells of the affected tissue. Such factors have been called "chalones" (*4*) but have not been isolated in pure form.

It has been suggested (*3, 5, 6*) that these factors are homeostatic mechanisms that serve to regulate proliferation of cells in normal tissues and that cancer cells have somehow escaped these normal control mechanisms. In this report we suggest that in a simple model of mitotic inhibition by diffusible factors, the competition between normal and cancer cells will strongly depend on the type of tissue involved and on the mechanism whereby the cancer cells escape regulation.

To make the arguments clear, let us adopt a simple model of inhibitor action. Assume that some or all cells in a tissue secrete inhibitor molecules and that (possibly other) cells in the tissue recognize the inhibitor concentration in their vicinity such that a high concentration will reversibly inhibit mitosis. A central question is what determines the inhibitor concentration near the responsive cells. In a tissue such as skin, inhibitors must be largely distributed by diffusion so that the local concentration will be determined by the nearby source (number of nearby secreting cells), by the diffusion properties and geometry of the tissue, and by the lifetime of the molecules against metabolic degradation or loss to the circulatory system. Such a tissue could respond to a local loss of secreting cells by local proliferation so that such a mechanism could regulate the local thickness of skin (*7*). Simple mathematical models (*8–10*) have confirmed this behavior. We shall say that in such tissues the control is local or diffusive.

Rather different considerations must determine the inhibitor concentration in such organs as the liver (*11*) or bone marrow (*12*), where cells throughout the tissue respond with increased proliferation to any substantial loss of tissue cells. In such tissues we assume that inhibitor molecules circulate throughout the body and that their concentration is more or less uniform in the tissue, being largely determined by their source strength (number of secreting cells in the whole tissue) and by their lifetime against metabolism and excretion. In such a tissue, we shall say that control is systemic. Simple mathematical models