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Evidence for poecilogony in *Pygospio elegans* (Polychaeta: Spionidae)

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ABSTRACT: The spionid polychaete *Pygospio elegans* displays more than one developmental mode. Larvae may develop directly, ingesting nurse eggs while brooded in capsules within the parental tube, or they may hatch early to feed in the plankton before settling. Asexual reproduction by architomic fragmentation also occurs. Geographically separated populations of *P. elegans* often display different life histories. Such a variable life history within a single species may be interpreted either as evidence of sibling speciation or of reproductive flexibility (poecilogony). Four populations from the English Channel were found to demonstrate differing life histories and were examined for morphological and genetic variability to determine whether *P. elegans* is in fact a cryptic species complex. Significant but minor inter-population polymorphisms were found in the distribution of branchiae and the extent of spoonlike hooded hooks. These externally polymorphic characters did not vary with relation to life history, and variation fell within the reported range for this species. Cellulose acetate electrophoresis was used to examine 10 allozyme loci, 5 of which were polymorphic. Overall, observed heterozygosity (H_o = 0.161) was lower than that expected under Hardy-Weinberg equilibrium (H_e = 0.228). Significant heterozygote deficiencies, detected at the *Est** and *Xdh** loci in all populations (except *Xdh** at Ryde Sand, Isle of Wight, UK), are discussed. *F*-statistics were used to examine patterns of genetic structuring among both separate and pooled populations. F_{ST} values at all polymorphic loci indicated a significant level of genetic differentiation between populations, most probably related to isolation by geographic distance. No direct relationship between life history and genetic structure could be detected. Overall genetic identity among the 4 populations was high (I = 0.977 to 0.992). Overall, populations displaying larval brooding did not appear to be reproductively isolated from populations displaying a fully planktonic larval mode. Present data support the hypothesis that *P. elegans* is poecilogonous.

KEY WORDS: Poecilogony · Life history · Allozymes · Polychaete · Spionidae · English Channel

INTRODUCTION

Pygospio elegans Claparède 1863 (Polychaeta: Spionidae) is a common component of boreal littoral benthic faunas. It has a circum-boreal distribution being recorded from the Arctic, Baltic, Northern Atlantic and Northern Pacific Oceans, the North and Othotsk Seas, the Mediterranean and the coast of South Africa (Clay 1967). It has wide habitat tolerances and may range from the lower superlittoral down to depths of 100 m (Refors 1933, Remane & Schlieper 1950, Gerdes & Krumbein 1985), tolerating salinities of 2‰ to hypersaline pools (Nicol 1935, Hempel 1957).

Pygospio elegans is one of a number of species to have evolved opportunistic life strategies to take advantage of the 'pioneer' niche: this group is able to rapidly re-colonise defaunated substrates (Grassle &

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Grassle 1974, McCall 1977). This first successional stage usually involves small 'r-selected' polychaetes (classically *Capitella capitata* and members of the family Spionidae: Grassle & Grassle 1974, McCall 1977, Noji & Noji 1991) able to establish dense populations swiftly and physico-chemically recondition the sediment (Gallagher et al. 1983, Reise 1983, Schmager-Noji 1988). Populations of *P. elegans* achieve densities of up to 10^5 ind. m^{-2} (Anger 1977, Trueblood et al. 1994, Morgan 1997), usually in response to conditions of organic enrichment. *P. elegans* is sedentary and constructs tubes approximately 1 mm in diameter. At high population densities 'tube-beds' may be formed. These biogenic structures may elevate the sediment surface by some tens of centimetres over areas of many square metres and have important effects on local ecology (Morgan 1997).

Pygospio elegans is dioecious and sexually polymorphic; mature males possess an extra set of branchiae on the second setiger. Unusually for a tube-dwelling spionid, fertilisation is preceded by copulation (authors' pers. obs., see also Schlötzer-Schrehardt 1991) and the female stores spermatophores in dorsal *receptacula seminalis* before releasing them for internal fertilisation (Hannerz 1956). Initial development proceeds in the coelom until, at a size of 100 µm, embryos and accessory yolk bodies (nurse eggs) are laid into external capsules extruded by the parent (Rasmussen 1973). Each such capsule contains the product of one gametogenic setiger. Capsules are attached to each other in a sling, and to the inside of the tube by single stalks. Here larvae complete the benthic stage of their development.

Geographically separated populations of *Pygospio elegans* can display different life histories (Rasmussen 1973, Gudmundsson 1985). Larvae may develop directly within brood capsules, ingesting nurse eggs (adelphophagia, a type of lecithotrophy), or they may hatch at an early stage, without adelphophagia, to feed in the plankton before settling (Hannerz 1956). Furthermore, asexual reproduction may occur by architomic fragmentation into many 3- to 4-setiger lengths; fragments rapidly regenerate at either end and a complete new adult may be formed in 8 d at 20°C (Rasmussen 1953). Asexual reproduction appears to be controlled, at least in part, by conditions of food availability and conspecific density (Wilson 1985). The occurrence of more than one developmental mode in a single species—poecilogony—is an adaptation reported largely from opportunistic species dwelling in disturbed benthic habitats (Chia et al. 1996), and it appears to be restricted to mud-flat-dwelling polychaetes and molluscs (Hoagland & Robertson 1988, Bouchet 1989). Poecilogony has been demonstrated in other spionid polychaetes (e.g. *Streblospio benedicti*, Levin et al. 1991, Levin & Bridges 1994, and *Boccardia proboscidia*, Gibson 1997). Poecilogony has been seen as a potential mechanism for adaptive response to fluctuating environmental conditions, offering the potential for both dispersion and local proliferation (Blake & Kudenov 1981).

In many instances the existence of more than one reproductive mode has been re-interpreted as evidence of sibling speciation rather than of phenotypic flexibility (Hoagland & Robertson 1988). Interest and speculation regarding the taxonomic status of *Pygospio elegans* has been aroused by its wide habitat tolerance and seemingly flexible life history, particularly as it may be a useful pollution indicator species (Anger 1977). Putative sibling species have been uncovered in other spionids demonstrating varying phenotypic traits, e.g. *Polydora ciliata* (Mustaquim 1988), *Marenzellaria viridis* (Bastrop et al. 1995), *Polydora ligni* (Rice & Simon 1980, Rice 1991), *Streblospio benedicti* (Rice 1991) and the opportunistic capitellid *Capitella* sp. (Grassle & Grassle 1976). An experiment by Anger (1984) to examine the hypothesis of poecilogony in *Pygospio elegans* involved culturing individuals from 3 geographically separate populations. One, sampled from an organically polluted site in Kiel Bay in the Baltic, was very unusual in that it reproduced exclusively asexually, apparently having undergone an extreme physiological adaptation and having lost the ability to produce gonads. The other 2 study populations produced planktonic larvae only. Anger (1984) found that reproductive mode was conserved in the face of changes in temperature and salinity. Morgan (1997) performed a similar experiment involving populations from the Somme Bay (Northern France) and Ryde Sand (Isle of Wight, UK), which were known to show differing life histories. Conditions of density and nutrient supply were varied, and results again indicated conservation of larval developmental mode in both populations (Morgan 1997). It is of course possible that the factors varied in the above experiments simply had no role in determining developmental mode, or that they were not varied sufficiently, or at the correct time in the life cycle to have an effect. However, these experiments suggest that *P. elegans* cannot make adaptive changes in life history within a population. Hoagland & Robertson (1988) defined poecilogony as a developmental polymorphism manifested (1) geographically, (2) seasonally or (3) in response to environmental stimuli. The results of Anger (1984) and Morgan (1997) do not support the hypothesis of poecilogony given criterion (3). Anger (1984) postulated cryptic speciation on the basis of her data and called for further investigation into *P. elegans* to include comparative studies of morphology, reproductive biology and population genetics. Hoagland & Robertson (1988)

Table 1. *Pygospio elegans*. Life history of populations at each study site. L: lecithotrophic; P: planktotrophic

Location	Collection date (1997)	No. of females carrying offspring	Larval characteristics				Frequency of asexual reproduction
			Yolk density per larva	Swimming setae	Capsular density	Devel. mode	
Plym Estuary	27 Jan	49	High (100%)	No (100%)	2–3	L	0%
Ryde Sand	30 Oct	30	High (100%)	No (100%)	2	L	0%
Swale Bay	13 Mar	45	Low (100%)	Yes (100%)	15–22	P	0%
Somme Bay	22 Feb	96	Low (100%)	Yes (100%)	18–23	P	0%

noted the importance of genetic data in testing claims of poecilogony at a geographic level. This paper responds to this call and presents new morphological, population genetic and life-history data for *P. elegans* to assess the relationship between life-history variation and taxonomic status. This species thus joins the short list of apparently poecilogonous polychaetes to be examined at a genetic level for cryptic speciation (see also Grassle & Grassle 1976 on the *Capitella* complex; Rice & Levin in press, on *Streblospio gynobranchiata*).

METHOD

Pygospio elegans was studied at 4 localities on the English Channel coast: the Plym Estuary, Devon, UK (50° 23' N, 4° 10' W), Ryde Sand, Isle of Wight, UK (50° 44' N, 1° 10' W), Swale Bay, Kent, UK (51° 20' N, 0° 53' E), and Somme Bay, Picardie, France (50° 14' N, 1° 33' E; Fig. 1). These sites were chosen following a survey to find readily available populations which displayed differing life histories for comparison. Individuals were collected and examined for larval developmental mode and evidence of asexual reproduction. The null hypothesis, of conspecific status among these 4 populations, was then tested through comparison of morphological and genetic characters. Dates of sample collection are detailed in Table 1.

Swale Bay is tidal and subject to strong hydrodynamic conditions. *Pygospio elegans* here appears limited in distribution to seaward zones stabilised by *Zostera marina* and *Mytilus edulis*, where it occurs in small patches in densities of 10 to 1000 ind. m^{-2}. This population is known to experience large fluctuations in density (authors' pers. obs.). Somme Bay is a macrotidal estuary with an intertidal zone 72 km^2 in area and a maximum tidal amplitude of 9 to 10 m. A double low water enforces a highly dynamic erosional and depositional regime. Macrobenthic surveys throughout the 1980s and 1990s have recorded periodic mass mortalities attributed to anoxia promoted by high summer temperatures and organic enrichment (Rybarczyk et al. 1996). Although *P. elegans* proliferated during periods of disturbance, population densities fluctuated widely (Desprez et al. 1992); maximum recorded density in Somme Bay is 600 000 ind. m^{-2} (Morgan 1997). Plym Estuary is characterised by low hydrodynamic disturbance and high organic input and hosts a very patchily distributed *P. elegans* population. Ryde Sand on the Isle of Wight is located near a waste outfall and is an organically enriched and productive site. It is moderately exposed to wave action. Here *P. elegans* maintains low densities.

Sediment containing *Pygospio elegans* was identified by the protrusion by a few millimetres of small tubes (1 mm diameter) at the surface. *P. elegans* occurred intertidally at each site, and was sampled by manual coring over a large area. Tubes containing individuals at all stages of development were retained on a 0.5 mm mesh.

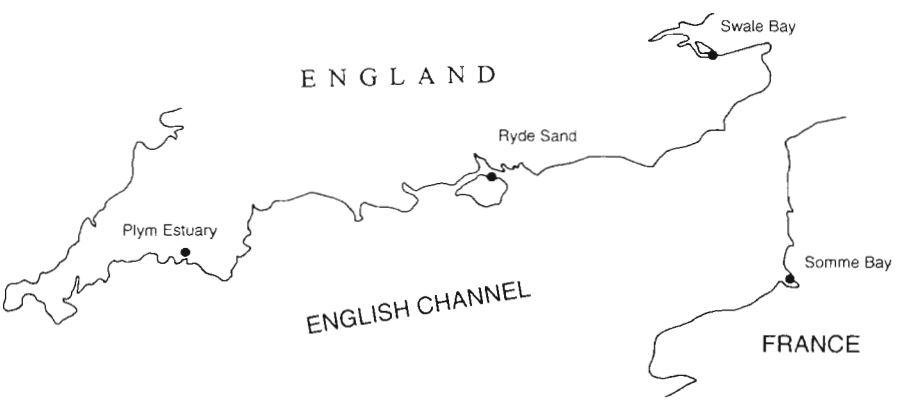

Fig. 1. Sites of populations of *Pygospio elegans* sampled during the present study

Table 2. *Pygospio elegans*. Enzyme systems and loci examined, and optimal electrophoretic run conditions. Buffers used were CP (0.01M citrate-phosphate, pH 6.4), and TG (0.025M tris-glycine, pH 8.5), as specified in Richardson et al. (1986)

Enzyme	E.C. number	Locus	Buffer system	Run time (min)
Esterase	3.1.1.1	*Est**	CP	15
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pdh**	TG	15
Glucose dehydrogenase	1.1.1.47	*Gldh**	TG	20
Hexokinase	2.7.1.1	*Hk**	TG	15
Malate dehydrogenase	1.1.1.37	*Mdh-1, Mdh-2**	CP	25
Phophoglucose isomerase	5.3.1.9	*Pgi**	CP	50
Phosphoglucomutase	5.4.2.2	*Pgm-1, Pgm-2**	CP	50
Xanthine dehydrogenase	1.2.3.2	*Xdh**	TG	25

Morphological analysis. In the laboratory all live worms collected were removed from their tubes, and placed in standing seawater tanks for up to 3 d, during which time all gut contents were voided. All individual females in samples bearing embryos and larvae in external capsules were examined to determine the developmental mode of their offspring. Appearance of offspring was recorded. Larvae destined for early release into the plankton were characterised by their small size and the early development of 'parachute' setae. In contrast, directly developing larvae appeared 'hunchbacked' and engorged with dense orange yolk, and occur in lower numbers per capsule. Any asexually reproducing individuals were noted, using the description of Rasmussen (1953) as a reference.

A random subset of live individuals from each population was examined morphologically. *Pygospio elegans* is sexually polymorphic and only non-juvenile (total setiger number >30), female specimens were measured to avoid introducing age- or sex-based variation into the data. A review of previous morphological descriptions of *P. elegans* (Day 1967, Foster 1971, Light 1977) informed the selection of a set of potentially polymorphic characters for measurement: in particular, the extent and distribution (1) of dorsal branchiation and (2) of those anterior, ventral hooded hooks described in *P. elegans* as 'spoonlike'. Measurements were made of total number of setigers, first and last setigers on which dorsal branchiae occurred, first and last setigers on which anterior, 'spoonlike' hooded hooks occurred, number of such spoonlike hooks per setiger, and the shapes of the prostomium (anteriormost setiger) and pygidium (posteriormost setiger).

Biochemical genetic analysis. A set of biochemical genetic markers (allozymes) were developed using cellulose acetate electrophoresis to investigate the genotypic divergence between populations of *Pygospio elegans*. After morphological examination specimens were individually frozen at −70°C and stored until genetic analysis. Thus life-history data and genetic data were gathered from the same individuals. Prior to electrophoresis, specimens were individually placed into 1.5 ml centrifuge tubes with 10 µl grinding buffer (0.1M Tris/HCl, pH 8.0, with 10 mg ml^{-1} sucrose), and homogenised whole, over ice, using a motorised micropestle. Extract was then centrifuged at 14 000 rpm (16 000 × *g*) at 4°C for 10 min. All electrophoretic runs used 'Helena Titan III'™ cellulose acetate plates. Extract was loaded onto each plate up to 6 times per individual to compensate for the low concentrations of enzymes yielded by these small polychaetes (approximate wet weight = 0.5 mg). All runs took place at 200 V, over ice. Eight enzyme systems, representing 10 putative genetic loci, were studied using 2 buffer systems (Table 2) and visualised using histochemical stains (all chemicals from Sigma Chemical Co. Ltd, Poole, Dorset, UK) modified from Richardson et al. (1986) and Hebert & Beaton (1989). Five further enzyme loci were visualised (*Adh-1**, *Adh-2**, E.C 1.1.1; *Idh-1**, *Idh-2**, E.C. 1.1.1.42; *Iddh**, E.C. 1.1.1.14: 1 locus) but their resolution was compromised by low concentration and activity, and so were not scored. Where more than 1 locus coded for a single enzyme system, loci were labelled in order of increasing mobility. Alleles were labelled by their mobility relative to the most common allele present in all populations (designated a mobility of 1.00).

Genetic data analysis. A set of indices of genetic variability were determined from allele frequencies of *Pygospio elegans* for each population. The mean number of alleles per locus and the percentage of polymorphic loci were calculated. The distribution of genotypes (observed heterozygosity) was tested for conformity to Hardy-Weinberg equilibrium using χ^2 tests with Levene's (1949) correction for small sample size and Yate's correction for continuity (Elston & Forthofer 1977). Wright's (1951, 1965) fixation index (F) was also determined: this deviates from zero in relation to the deviation of observed heterozygosity from Hardy-Weinberg expectations. The derived F-statistics F_{IS} and F_{ST}, were also calculated. F_{IS} is defined as the correlation of homologous alleles with

reference to the local population assuming random mating; significance of the deviation of F_{IS} from zero was calculated using the chi-squared test of Li & Horvitz (1953):

$$\chi^2 = F_{IS}^2\, N(k-1), \text{ where degrees of freedom} = k(k-1)/2,$$

where N is the number of individuals sampled and k is the number of alleles present at the locus. F_{ST}, a measure of the genetic differentiation between populations, increases from 0 to 1 with increasing interpopulation differentiation. Significance of the departure from zero of F_{ST} was calculated using the formula of Workman & Niswander (1970):

$$\chi^2 = 2\, N F_{ST}(k-1), \text{ where degrees of freedom} = (k-1)(s-1)$$

where s represents the number of populations compared. Significance levels of F_{IS}, F_{ST} and of departure from Hardy-Weinberg equilibrium were adjusted for multiple tests using the sequential Bonferroni technique (Rice 1989).

An estimate of the number of migrants per deme per generation (N_em), i.e. the level of gene-flow between populations, may be calculated from F_{ST} using the formula

$$N_em = ([1/F_{ST}] - 1)/4$$

The critical value of N_em is 1 (Slatkin 1985); a value of N_em exceeding this theoretically indicates a level of gene-flow between populations sufficient to offset the effects of random genetic drift. Gene-flow is assumed to result from dispersal from a central pool of migrants (island model).

Nei's (1972) indices of genetic identity (I) and genetic distance (D) were calculated to estimate overall genetic differentiation between populations and test the null hypothesis of conspecific status. Cluster analysis, based on Nei's (1978) unbiased genetic distance, was then performed using the unweighted pair group arithmetical average (UPGMA) method, to produce a dendrogram of population similarities. Calculations of allele and genotype frequencies, conformity to Hardy-Weinberg equilibrium, F, F_{IS} and F_{ST}, I and D and the UPGMA cluster analysis were performed using BIOSYS 1, version 1.7 (Swofford & Selander 1989).

RESULTS

Life history

Details of the larval developmental mode in each population are given in Table 1. Each population appeared to demonstrate only 1 larval developmental mode. Planktotrophic larvae, equipped with long swimming setae and present in large numbers (range: 15 to 23 ind. per capsule) were observed from the Somme Bay and Swale Bay populations. Lecithotrophic larvae, lacking long setae and conspicuously dense with yolk, were found brooded in capsules of 2 to 3 larvae each in the Ryde Sand and Plym Estuary populations. No asexually reproducing individuals were discovered in any sample from any population. Some individuals (<5% overall) were noted regenerating only either a head or a tail end but these were deemed the victims of predation or some other physical damage. Asexually reproducing individuals would have appeared to be regenerating both head and tail from a short, adult-sized body fragment (Rasmussen 1953). Such fragments, though small, would have been found within tubes (Gudmundsson 1985) and it is not thought that any were lost through sieving at 0.5 mm.

External morphology

No variation in prostomial or pygidial shape was observed and all specimens showed characteristics typical of the species. Table 3 displays further morphological data. Variation in the first presence of branchiae was found within 3 populations; however only the Ryde Sand population differed significantly, showing the most posterior commencement of branchiation ($p < 0.001$). Branchiae commenced over the range of the 12th to 14th setigers overall. A weak but signifi-

Table 3. *Pygospio elegans*. Comparison of externally polymorphic features of polychaetes from different geographical locations. n = number of females examined. Frequency of observation is shown in parentheses

Location	n	First branchiate setiger	Mean proportion of setigers carrying branchiae ± SD	No. of setigers carrying 'spoonlike' hooded hooks
Swale Estuary	20	12 (75%), 13 (25%)	0.47 ± 0.05	5 (95%), 6 (5%)
Ryde Sand	20	13 (90%), 14 (10%)	0.41 ± 0.05	5 (80%), 6 (20%)
Plym Estuary	20	12 (100%)	0.45 ± 0.09	4 (10%), 5 (90%)
Somme Bay	20	12 (90%), 13 (10%)	0.52 ± 0.07	5 (100%)

cant overall relationship with total setiger number was found ($r = 0.36$, $p < 0.001$). Overall the number of branchiate setigers correlated well with the total setiger number among females ($r = 0.82$, $p < 0.001$). However, the relationship between branchial displacement and somatic size was found to show some degree of polymorphism: a significant difference in the ratio of branchiated setigers to total setigers was found between populations (ANOVA, $p < 0.001$). This ratio was lowest at Ryde Sand (0.41 ± 0.05, mean ± SD) and highest at Somme Bay (0.52 ± 0.07). Differences in this ratio were mainly affected by the number of posterior setigers lacking branchiae. All individuals from all populations carried hooded hooks from the 8th setiger and then posteriorly to the end. A weak but significant positive overall correlation ($r = 0.40$, $p < 0.001$) was noted between total setiger number and the number of setigers carrying spoonlike hooded hooks. The distribution of the spoonlike hooded hooks showed no significant variation among the Somme Bay, Ryde Sand and Swale Estuary populations; individuals from the Plym Estuary population had fewer spoonlike hooks, however ($p < 0.001$).

Table 4. *Pygospio elegans*. Allele frequencies per locus for each polychaete population. Relative mobilities of alleles shown in parentheses. n = number of individuals sampled

Locus	Allele	Population			
		Plym	Ryde	Somme	Swale
Est•		n = 90	n = 70	n = 85	n = 94
	A (1.06)	0.122	0.036	0.018	0.005
	B (1.03)	0.194	0.200	0.176	0.229
	C (1.00)	0.433	0.714	0.729	0.697
	D (0.97)	0.250	0.050	0.076	0.069
G6pdh•		n = 90	n = 70	n = 92	n = 93
	A (1.50)	0.311	0.150	0.277	0.048
	B (1.00)	0.689	0.850	0.723	0.952
Gldh•		n = 50	n = 10	n = 50	n = 50
	A (1.00)	1.000	1.000	1.000	1.000
Hk•		n = 72	n = 64	n = 79	n = 60
	A (1.25)	0.090	0.086	0.108	–
	B (1.11)	0.194	0.133	0.158	0.092
	C (1.00)	0.646	0.734	0.722	0.875
	D (0.90)	0.069	0.047	0.013	0.008
	E (0.76)	–	–	–	0.025
Mdh-1•		n = 50	n = 10	n = 50	n = 50
	A (1.00)	1.000	1.000	1.000	1.000
Mdh-2•		n = 50	n = 10	n = 50	n = 50
	A (1.00)	1.000	1.000	1.000	1.000
Pgi•		n = 89	n = 70	n = 100	n = 94
	A (1.70)	–	–	–	0.005
	B (1.33)	0.022	0.029	0.065	0.021
	C (1.25)	0.096	0.114	0.125	0.069
	D (1.00)	0.809	0.693	0.695	0.782
	E (0.75)	0.051	0.114	0.055	0.064
	F (0.67)	0.022	0.050	0.060	0.059
Pgm-1•		n = 50	n = 10	n = 50	n = 50
	A (1.00)	1.000	1.000	1.000	1.000
Pgm-2•		n = 50	n = 10	n = 50	n = 50
	A (1.00)	1.000	1.000	1.000	1.000
Xdh•		n = 78	n = 61	n = 82	n = 84
	A (1.07)	0.058	0.057	0.055	0.220
	B (1.00)	0.603	0.475	0.829	0.518
	C (0.90)	0.333	0.262	0.085	0.250
	D (0.78)	0.006	0.205	0.030	0.012

Population genetic data

Allele frequencies were generated for all examined loci (Table 4). Private alleles at the *Hk*• and *Pgi*• loci were detected in the Swale Bay population, although at very low frequencies. The Swale Bay population also lacked an allele at the *Hk*• locus that was shared by the other 3 populations. Polymorphisms were apparent in all populations at the same 5 loci. Observed heterozygosity (H_o) within the populations ranged between 0.126 and 0.192 (Table 5). Observed heterozygosity was lower than expected heterozygosity according to Hardy-Weinberg equilibrium. An analysis of fixation indices for each variable locus showed that the main sources of this deficiency were the *Est*• and *Xdh*• loci (Table 6), which both showed highly significant positive deviations from zero (except in the case of *Xdh* at Ryde Sand).

Variations in F_{IS} and F_{ST} for populations taken separately are shown in Table 7. F_{ST}, the estimate of the

Table 5. *Pygospio elegans*. Genetic variability measurements for polychaete populations (SE shown in parentheses). A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99. Expected heterozygosity shows an unbiased estimate (see Nei 1978)

	Mean sample size per locus	Mean no.of alleles per locus	% polymorphic loci	Mean heterozygosity	
				Observed (H_o)	Expected (H_e)
Plym Estuary	66.9 (5.9)	2.4 (0.5)	50.0	0.149 (0.063)	0.253 (0.089)
Ryde Sand	38.5 (9.5)	2.4 (0.5)	50.0	0.192 (0.074)	0.230 (0.083)
Somme Bay	68.8 (6.5)	2.4 (0.5)	50.0	0.173 (0.068)	0.208 (0.071)
Swale Bay	67.5 (6.6)	2.5 (0.6)	50.0	0.126 (0.047)	0.178 (0.074)
Pooled	242.7 (28.3)	2.6 (0.6)	50.0	0.161 (0.058)	0.228 (0.079)

Table 6. *Pygospio elegans*. Wright's (1951, 1965) fixation index (F) for variable loci in each polychaete population. Adjusted significance of the deviation from Hardy-Weinberg expectations: $^{*}p < 0.05$, $^{**}p < 0.01$

Locus	Plym Estuary	Ryde Sand	Somme Bay	Swale Bay
*Est**	0.222**	0.680**	0.481**	0.349*
*Hk**	−0.555	−0.009	0.028	−0.110
*G6pdh**	−0.235	−0.064	−0.221	0.183
*Pgi**	0.055	0.068	0.021	0.094
*Xdh**	0.755**	0.081	0.635**	0.520**

Table 7. *Pygospio elegans*. F_{IS} (estimate of the correlation of homologous alleles between individuals within populations) and F_{ST} (estimate of the variance of allele frequencies between populations) for populations taken separately. Adjusted significance levels: $^{*}p < 0.05$; $^{**}p < 0.01$. N_em (estimate of number of migrants per deme per generation) calculated from F_{ST}

Locus	F_{IS}	F_{ST}
*Est**	0.618**	0.045**
*Hk**	−0.024	0.026**
*G6pdh**	−0.032	0.069**
*Pgi**	0.058	0.010**
*Xdh**	0.457**	0.069**
Mean	0.259**	0.044**
N_em	5.43	

Table 8. *Pygospio elegans*. Nei's (1972) genetic identity (I). Pairwise comparisons of all populations

	Plym	Ryde	Somme	Swale
Plym	–	0.982	0.983	0.977
Ryde		–	0.985	0.995
Somme			–	0.981
Swale				–

variance of allele frequencies between populations, was significant for individual populations at all polymorphic loci (adjusted $p < 0.01$) and varied from 0.010 to 0.069, indicating a degree of genetic structuring among the populations.

Nei's (1972) index of genetic similarity lay within the range $I = 0.977$ to 0.992 (Table 8), indicating the high level of genetic relatedness of the English Channel populations. Cluster analysis yielded a UPGMA dendrogram in which the Plym Estuary population appeared most dissimilar; however, the overall level of similarity was very high (Fig. 2). It is notable that the most similar populations—at Ryde Sand and Swale Bay—had contrasting life histories.

DISCUSSION

Life history variability

Previous studies of *Pygospio elegans* have described location-specific reproductive behaviours. It is debatable as to whether these observations were indicative of poecilogony. Gudmundsson (1985) showed that the life history of *P. elegans* at Cullercoats, NE England, involved entirely benthonic larval development, each brood-capsule containing only 2 directly developing larvae which left the brood capsule at the 14 to 20 setiger stage. This contrasted with an adjacent population at Blyth which produced both planktonic and benthonic larvae. Two sites on the Jutland peninsula (Denmark) studied by Rasmussen (1973) also yielded contrasting populations. At Isefjord (Sjaelland peninsula: Denmark), planktonic and benthonic larvae were produced simultaneously and asexuals were absent. At Horsen's Fjord (Jutland peninsula), however, the population first produced only planktonic larvae, and then entirely benthonic larvae, whilst asexual fragmentation occurred throughout. It is difficult to assess gene-flow between these 2 sites, which are separated by approximately 120 km across the Kattegat, with the

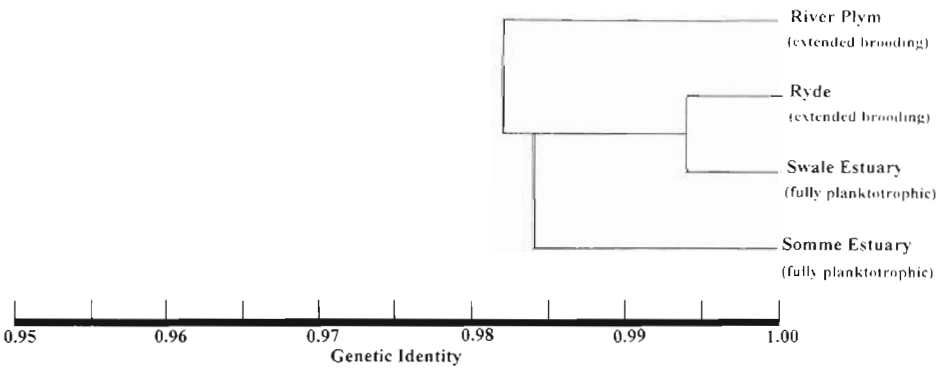

Fig. 2. *Pygospio elegans*. UPGMA dendrogram of genetic similarities based on Nei's (1978) unbiased genetic distance. Life history of each population is superimposed

island of Samsø intervening. Rasmussen (1973) noted no environmental differences between these sites.

Similar variability in reproductive activity has been found among the 4 English Channel populations investigated during the present study (Table 1). Observations on available young-bearing females indicated that each population demonstrated only 1 larval developmental mode at the time samples were taken for genetic and morphological analysis. It is possible that larval developmental mode varies temporally in a population. The Somme Bay population has been studied over an extended period, and it demonstrated exclusively planktonic development during 1990 to 1997 with no asexual activity (Morgan 1997). Lecithotrophic development was also observed by Morgan (1997) at Ryde Sand from samples taken in November 1996. Repeat visits to the Plym Estuary and Swale Bay during June and July of 1997 also served to verify the observed life histories reported here (authors' pers. obs.). Spatial replication of sampling at each site was extensive and it is unlikely that any large- to medium-scale patch variation in life history was overlooked at any site.

Morphological variability

Prostomial shape is known to display some variation within the genus *Pygospio*: *P. californica* has a non-incised, pointed prostomium (Hartman 1936). The 4-lobed pygidial shape is diagnostic of *Pygospio elegans* (Claparède 1863). No interpopulation variation in prostomial or pygidial shape were noted from present observations.

Evidence for widespread variation in branchial displacement is present in the literature. Although all accounts agree on the general region in which the branchiae first appear anteriorly, some descriptions report branchiae of females continuing posteriorly for approximately 8 setigers only (Day 1967, Hartmann-Schröder 1971) while others describe branchiae in females occurring more extensively along the body (Foster 1971). Significant variation in branchial displacement (first branchiate setiger; ratio of branchiate to total setigers) was noted between populations; such variation fell within the range reported in the literature. A positive allometric relationship between the number of branchiate setigers and somatic size was found, and apparent inter-population differences in branchial displacement may simply reflect somatic size differences. Such differences might be attributed to varying environmental conditions or temporal variation in sampling. Local conditions of oxygen availability could also be responsible. Branchiae are involved in gas exchange, and the more frequently immersed Ryde Sand population may have experienced less chronic anoxia than occurs on the mudflats of the Somme Bay, which can lay exposed to air for nearly three-quarters of the tidal cycle. The greatest difference was found between the populations of Ryde Sand and Somme Bay. Intertidal sediments at Ryde Sand were also coarser than at Somme Bay, leading to better penetration of oxygenated water and better drainage during low tide. It should be noted that the character of branchial distribution may be rendered unreliable by the species' ability to regenerate lost extremities. The extent of such regeneration among individuals is unknowable. Further work is needed to determine whether regenerated setigers restore the animal faithfully to its former state and size, or whether regenerated sections are different, for example entirely lacking branchiae. If the latter case were true, then the average ratio of branchiate setigers to total setiger number must decrease, a situation which from present data would point to an increased regenerative rate in the Ryde Sand population compared with that of Somme Bay.

The characteristic, anterio-ventral 'spoonlike' hooded hooks were first described in *Pygospio elegans* by Söderström (1920) as normal hooded hooks with worn-away teeth; he thus ascribed them to mature individuals, and suggested that the number of such hooks might increase with age. Clearly, were this the case, the number of such hooks could not be taken as a reliable taxonomic character. However, observations made in the present study suggest that these spoonlike hooks are distinct structures, unrelated to the more posterior, bidentate hooded hooks. Spoonlike hooks have a distally thickened, rounded tip and lack a central fang. Apart from the notable structural differences, no gradient of wear was observed from the hindmost row of spoonlike hooks to the foremost row of bidentate hooded hooks: in all cases the transition of forms was abrupt from one setiger to the next. However, the very weakly significant positive correlation between total setiger number and number of setigers carrying spoonlike hooks potentially supports Söderström's (1920) contention that age is involved. Since growth by setiger addition occurs at the posterior end, extra spoonlike-hook-bearing setigers could not be interposed during thoracic growth to produce this result, however. Hooded hooks are perhaps shed and replaced by spoonlike hooks in setigers 8 onwards as the animal grows.

The present results are conservatively interpreted as showing no significant morphological evidence for species divergence among the populations studied. Rather, a natural continuous variation within characteristics is apparent. Studies which have defined new species of *Pygospio* have done so on the basis of far

more dramatic morphological differences. For example, *P. californica* Hartman, 1936, has a non-incised, pointed prostomium, hooded hooks starting from setiger 23 and branchiae commencing on setiger 19.

Genetic variability

The present allozyme data emphasise the similarity between these populations. Thorpe (1983) showed that Nei's (1972) genetic identity, *I*, among conspecific populations exceeded 0.95 in 80% of cases. Present calculations of *I*, based on examination of 10 loci, thus suggest that the populations examined in this study were conspecific ($I \geq 0.977$; see Table 8). The possibility remains that the genetic divergence at the examined loci is not representative of those parts of the genome involved in reproductive isolation. However, there is no evidence for cryptic speciation from the loci available for investigation through allozyme electrophoresis.

Few previous studies have reported levels of genetic differentiation or identity between populations of polychaetes. Rice & Simon (1980) conducted allozyme electrophoresis on allopatric populations of the spionid *Polydora ligni* and found 1 population which appeared genetically distinct from the others, with *I* lying in the range 0.5878 to 0.6094. It was suggested that this may represent a discrete species. Cadman & Nelson-Smith (1990) examined phenotypically different populations of *Arenicola marina* and discovered an *I* value of 0.2717; a taxonomic revision of the species followed (Cadman & Nelson-Smith 1993). Debate persists about the relationship between genetic variation and speciation and is fuelled by discoveries of groups in which speciation proceeds with little genetic differentiation (African lake cichlids and birds; Thorpe 1983, Meyer et al. 1990). However, these are exceptional cases, and identity measures based on genetic characters such as Nei's (1972) *I* have been widely used to assess the taxonomic relationships of organisms for which reliable taxonomic characters are not available (e.g. Rogers et al. 1995).

Anger et al. (1986) determined the mean planktonic period of larval development in *Pygospio elegans* larvae, hatched at 3 setigers in the laboratory, at 20 to 30 d at 18°C, and 60 to 70 d at 6°C. There is therefore potential for planktonic larval mediated dispersal in this species. The level of gene-flow between populations, i.e. immigration of planktonic larvae from the Somme and Swale Bays to the Plym Estuary and Ryde Sand, was sufficient to maintain overall genetic homogeneity ($N_e m = 5.43$). Despite this, however, the significance of F_{ST} at all polymorphic loci suggested a degree of structuring within the English Channel metapopulation (Table 7). This structuring may have arisen through gene-flow insufficient to counteract genetic drift (despite $N_e m > 1$), or through selection. Although effective population size (N_e) is unknown for these 4 populations, observation of extreme density fluctuations at these sites (authors' pers. obs.) suggests that N_e may be low and may lead to a high level of genetic drift.

The hypothesis that genetic structuring was related to geographical distance between populations was examined. Measurement of long-shore distances between sites showed that Ryde Sand lay approximately equidistant between Plym Estuary and Swale Bay (see Fig. 1). However, the UPGMA dendrogram (Fig. 2) showed unequal levels of similarity between these sites, instead suggesting a 'stepping-stone' model of gene-flow between the Ryde Sand and Swale Bay populations, with isolation of the Plym Estuary, perhaps through some hydrographic barrier to gene-flow. Somme Bay, although geographically closer to Ryde Sand than was Swale Bay, was less genetically similar, suggesting attenuation of gene-flow from France across the open water of the English Channel to the southern coast. This would be expected from hydrographic models of the area which show net transport to the North Sea (Salomon & Breton 1993).

There was no correlation between the level of genetic structuring inferred from F_{ST} and variation in reproductive strategy, further supporting the hypothesis that the English Channel *Pygospio elegans* metapopulation is poecilogonous. Variation in life history may therefore be a response to local environmental conditions, as proposed by Rasmussen (1973).

Heterozygote deficiency is commonly found in studies of marine invertebrates (Singh & Green 1984, Zouros & Foltz 1984, Creasey et al. 1996) and significant heterozygote deficiencies were detected in *Pygospio elegans* at the *Xdh** and *Est** loci (Table 7; F_{IS} for all populations taken separately: 0.494 and 0.635 respectively, $p < 0.01$). Observed heterozygote deficiency may result from (1) mis-scoring of gels though the presence of null alleles, (2) bottlenecks in population size, and founder effects, (3) selection against heterozygotes, or for a specific allele, and (4) the Wahlund effect. Null alleles appear to be rare (Creasey et al. 1997). *Pygospio elegans* exhibits typical opportunistic behaviour, and population densities may fluctuate dramatically in space and time (Morgan 1997), potentially resulting in bottlenecks and founder effects. As a consequence, temporal variation in allele frequencies may be high, rare alleles may be more readily lost and heterozygosity may decrease (Nei et al. 1975). However, such an effect would be expected to affect all loci equally and this explanation is therefore unlikely in this instance. It is more likely that the heterozygote deficiencies at the *Xdh** and *Est** loci are

explained by selection for specific alleles. Many studies have demonstrated environmental selection pressures that affect heterozygosity in natural populations. Monti et al. (1986) showed temporal variation in heterozygosity at the *Mdh** locus in the bivalve *Ruditapes decussatus* driven by changes in temperature and hypoxia. Jollivet et al. (1995) found genetic polymorphism in alvinellid polychaetes living near hydrothermal vents and related this to differential allelic fitness to temperature. Creasey et al. (unpubl.) have shown reduced heterozygosity in crustacean populations in the vicinity of an oxygen minimum zone.

Present morphological and genetic data do not allow the rejection of the null hypothesis that the 4 English Channel populations are conspecific. These data, taken with the observation of variation in life histories, support the hypothesis that *Pygospio elegans* demonstrates poecilogony. A full consideration of the mechanisms by which poecilogony operates is beyond the scope of this paper, but it seems clear that larval developmental mode is influenced by the distribution of nurse eggs in external brood capsules. Nurse egg provision may be genetically predetermined or under physiological control, and therefore potentially adaptive. However, the ratio of true eggs to nurse eggs could be limited by the availability of sperm to fertilise oocytes, with potentially maladaptive consequences. A study of ovary ultrastructure may elucidate vittelogenic mechanisms underlying a control of nutrient supply to developing embryos and therefore a control of the ratio of true eggs to nurse eggs. Experiments are also required to determine the stage at which the future developmental mode of offspring is set during the life cycle of a poecilogonous individual.

Rapid exploitation of disturbed, often highly productive areas by 'pioneer' species may lead to the foundation and proliferation of dense colonies with short generation times and a non-dispersive larval mode, or even asexual proliferation. Pioneering populations of opportunists that become established in previously uncolonised areas, perhaps after introduction via ballast water, push forward the geographical range of the species but may be exposed to new selection pressures, leading to genetic isolation and movement along new evolutionary pathways. Bellan (1977) has described polychaetes as currently undergoing a 'full evolutionary phase' and it is perhaps unsurprising that so many instances of sibling speciation are now being proven with the advent of techniques to test for molecular polymorphisms. *Pygospio elegans*, like many other opportunists, is supposedly a circum-boreal species. The suspicion that this species in fact comprises a cryptic complex appears from the present data to be unfounded, at least on the geographical scale of the English Channel.

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