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Heterogeneous population growth, parental effects and genotype–environment interactions of a marine oligochaete

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Abstract Cultures of asexually reproducing populations of the oligochaete *Paranais litoralis* (Müller) collected from six different patches (3 to 50 m apart) on an intertidal mud flat in Flax Pond, New York, on two occasions, June and October 1993, showed significant differences among lines in life span, number of offspring produced, and in finite rate of increase (λ). Although growth rates were significantly lower in October than in June, they were always positive ($\lambda > 1$) in the laboratory cultures reared in field-collected sediment, while field data show that the densities of *P. litoralis* decreased sharply in summer and autumn from a seasonal high in early June. Cultures of worms reared at high densities without renewal of sediment crashed, and effects on individuals were irreversible: worms from late (declining) stages of population growth had a significantly higher mortality and lower reproduction than worms from earlier stages, also when transferred to high-quality food. Genetical analysis using RAPDs (random amplified polymorphic DNA) confirmed the existence of several clones of *P. litoralis* in our cultures. Experiments where parent and offspring were cultured in sediments of different qualities showed clone–environment interactions in the number of asexual offspring produced, but not in age at first reproduction. Clones also differed in that some showed significant parental effects of sediment quality on life-history characteristics while other clones did not. Our results indicate that *P. litoralis* populations in Flax Pond are not an example of a population subdivided into a set of permanent source and sink subpopulations, but rather an example of a continuously shifting mosaic of local growth conditions.

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Introduction

Natural populations are commonly not homogenous, but are built up of subpopulations with different growth rates, either due to intrinsic differences in growth characteristics, or simply because they are temporally out of phase with each other (May and Southwood 1990; Zajac and Whitlach 1991; Kawecki and Stearns 1993). If some areas support higher growth rates than others, and migration between areas occurs, the population may be composed of source and sink subpopulations (Dias 1996), where the life history and demography of the population as a whole are adapted to conditions of the area of the source populations, but may be poorly adapted to the environment in the area of the sink populations (Pulliam 1988; Kawecki and Stearns 1993). Many marine benthic populations appear to have growth rates far from $\lambda = 1$, in spite of an apparent overall constancy of populations. Zajac and Whitlach (1991) suggested that this may be due to spatial and/or temporal heterogeneity in population growth, a heterogeneity that often is missed in experimental investigations.

In a species where individuals can disperse and where the habitat is heterogeneous, growth conditions may also differ between generations. In this study, we investigate maternal effects as defined by Falconer (1981, p 124): "Maternal effects are prenatal and postnatal influences, mainly nutritional, of the mother on her young...". Maternal effects may have consequences for population growth, in particular for source and sink populations. If the difference between growth in source and sink populations is only due to environmental differences, immigrants from the source population coming into the sink population would be demographically similar to the original individuals in the sink, or at least

the differences would disappear within one generation. Maternal effects may change this, as demographic differences among individuals may remain another generation, causing the sink population to be more "source-adapted" than in the absence of maternal effects. Maternal effects may therefore decrease the difference in population growth between source and sink populations if influx of individuals from the source population to the sink population is high. If migration rates are low from source to sink, maternal effects may be important in reducing the rate of adaptation to the sink condition.

If the study species is reproducing asexually, genotype–environment interactions are especially interesting, because they may be important for maintaining clonal diversity (Vrijenhoek 1990). It is surprising that different clones can be maintained in an area, because the clone best adapted to the local conditions should outcompete other clones, leading to the occurrence of only one clone in that area (Vrijenhoek 1984; Sebens and Thorne 1985; Hughes 1989). However, this seems not to be the usual case: often many clones co-occur (Vrijenhoek 1990; Avise 1994). It has been proposed that clones differ slightly in their competitive ability in different habitats, as a result of genotype–environment interactions (Williams 1975; Vrijenhoek 1984), preventing (or slowing down) competitive exclusion. Strong genotype–environment interactions may also contribute to source and sink population structure, in preventing source-population immigrants from growing well in sink populations.

Until the 1980s, investigations on clonal structure were hampered by the difficulty of distinguishing among different clones, but the recent progress in molecular genetic techniques has partly resolved this. RAPD (random amplified polymorphic DNA) has been suggested as a convenient and sensitive method for distinguishing among closely related individuals and clones (e.g., Grosberg et al. 1996).

In the present study, we investigated the population biology of *Paranais litoralis* (Müller), an oligochaete common in estuaries and intertidal mud flats in, e.g., North America, Europe and Asia (Giere and Pfannkuche 1982). *P. litoralis* generally has a pronounced yearly cycle: very low density populations in early spring grow rapidly to produce a population maximum in early summer, after which the populations crash and remain at low densities until the following spring (Giere and Pfannkuche 1982; Cheng et al. 1993). The worms are infaunal deposit feeders, ca. 5 mm long, and generally propagate by fission (new individuals are produced asexually at the tail end of the "mother") so they lack a planktonic larval stage, but adult worms have a limited dispersal capability through swimming (authors' personal observation). Sexually mature individuals have been reported from Europe (Giere and Pfannkuche 1982), but we found no evidence of sexual reproduction in our study or in previous studies at the site where this study was conducted (Levinton and Stewart 1988; Cheng et al. 1993; Martinez 1993).

In this investigation we address the following questions: (1) Is population growth rate of *Paranais litoralis* homogeneous among patches on an intertidal mud flat? (2) If growth rates are heterogeneous, do some places consistently support higher growth rates than others, or does this change with time? (3) If growth rates are heterogeneous, is this caused by sediment quality, by factors intrinsic to the worms, or by a combination thereof? Are there genotype–environment interactions and/or maternal (parental) effects in this species? (4) Do differences in population growth under low-density conditions remain during high-density conditions? (5) Can RAPD be used to distinguish among clones? If so, is more than one clone of *P. litoralis* present on the mud flat?

Materials and methods

Study area and sampling for density estimates in the field

The study area is an intertidal mud flat, surrounded by a *Spartina alterniflora* marsh, in Flax Pond, Long Island, New York, USA. Samples for population density estimation (18 samples per date, taken at randomly chosen spots) were collected on five dates in 1993, and on eleven dates in 1994 (see Fig. 2) with a cut-off syringe (inner diameter 25 mm) to a depth of 10 mm. Samples were preserved in 4% formalin, with Rose Bengal added. On three dates, (2 June, 15 August and 19 October 1993) samples were taken according to a spatially hierarchical scheme (see Table 1). Three sites were randomly chosen with a distance of 30 to 40 m apart. Within each site, three plots were chosen to be about 5 m apart. Within each plot, three patches were chosen 1 m apart, and within each patch four replicate cores were taken.

Laboratory cultures

Does growth of *Paranais litoralis* differ among patches, and do some patches continuously support higher growth rates than others? To investigate this, we used laboratory cultures of worms collected in Flax Pond on 6 June and 8 October 1993. On each date samples were taken from six patches (each < 1 m^2), 3 to 50 m apart. Two of these patches (B and C) were chosen because they represented a low and high density in the spatial sampling in June, while the other patches were chosen randomly. From each patch we isolated 12 worms (parental generation, PG), and transferred these individually to petri dishes (35 mm diameter) containing sediment from the patch where worms were collected. The offspring (filial generation, FG) were transferred individually to petri dishes with sediment from their parental patch added. Worms from patches B and C were also cultured reciprocally, i.e., worms from B in sediment from C and worms from C in sediment from B (Fig. 1a). We also cultured FG worms from patches A, D, E and F in sediment from either patch B or patch C (Fig. 1a), to test if growth rates would differ with different sediment origin. Cultures were kept in aerated water from Flax Pond at approximate ambient temperatures (20 °C in June, 13 °C in October) in a constant temperature room. We checked the cultures every 2 d in June, and every 2 to 4 d in October: each time the offspring were counted and removed, and water and sediment were changed. To mimic the sediment and water quality the worms would experience in the field, new samples of water and sediment (frozen and thawed before use) were collected from the field weekly. The worms from the parental generation were very variable in their reproduction, and therefore the analyses of growth rate of the cultures were not statistically balanced: 8 to 24 worms of the filial generation were followed during their life cycle (ca. 50 d in June, ca. 100 d in October) from each treatment combination.

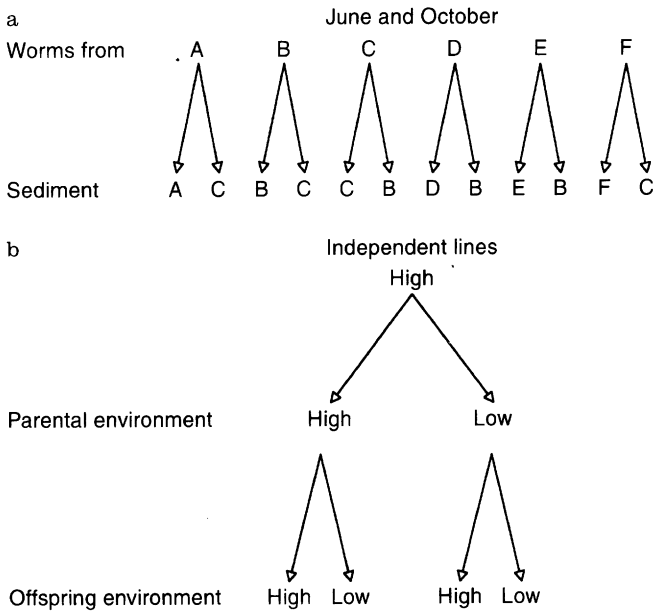


Fig. 1a, b Design of culture experiments. **a** Setup used for experiments in June and October 1993, investigating spatial and temporal variation in growth. **b** Setup used for experiments in May 1994, investigating parental effects and genotype–environment interactions

Population matrix analysis

Age-specific survival and fertility curves were used to construct birth–flow projection matrices (Caswell 1989) for each line–sediment combination, with 4 d as the projection interval. The corrected mean and confidence interval for the finite rate of increase (λ) were calculated with the accelerated bootstrap method (Dixon 1993), using a sample size of 2000. Differences in growth rate among cohorts were tested with randomization tests (Brault and Caswell 1993), with a procedure resembling a one-way ANOVA. This test compares the variance among groups in growth characteristics found in the experiment to a distribution of variances formed by constructing new groups by resampling (5000 iterations) from the original data set.

Elasticities (proportional sensitivities) of different matrix entries were calculated as described by Caswell (1989, pp 132 ff). The elasticity of an individual matrix element (such as the survival between two age-classes or the reproduction of a certain age-class) shows how a small change in that element would affect the overall population growth (λ) (see also "Discussion"). The differences in individual matrix entries among matrices within a season, and their contribution to variation in growth rate among projection matrices, were calculated as described in Brault and Caswell (1993). Contributions of matrix values to differences in growth rates between seasons (June and October) were calculated as described in Caswell (1989; see also Walls et al. 1991).

Maternal effects and genotype–environment interactions

Do some clones have generally higher growth rates irrespective of the environment, or are there genotype–environment interactions that contribute to growth differences? We addressed these questions with another set of cultures collected on 23 May 1994 from the same locations as described above (under "Laboratory cultures"). Here we include data from two lines maintained in the laboratory since collection in March 1994 (labeled L1 and L2), and three of the field lines collected in May (F1, F2 and F3). To reduce variation caused by parental environmental effects from previous, different field environments, all lines were started from single individuals cultured for 30 d prior to subsequent experiments. Newborn offspring from each line were cultured individually in sediment of either high or low quality (parental generation, PG). High quality sediment was spring sediment collected at Flax Pond; low quality sediment was three parts ashed sediments (organics burned off) to one part spring sediment. The offspring from the PG (filial generation, FG) were individually assigned into one of four treatments: (1) high–high, (2) high–low, (3) low–high, and (4) low–low quality sediment (Fig. 1b). The first treatment designation refers to the growth environment of the PG, and the second designation refers to the FG growth environment. Worms were checked (every 2 to 3 d) for 18 d to determine age at first reproduction (budding of offspring) and total number of offspring produced during this time interval. Ten individuals from each line were raised in each of the four treatments.

RAPD analysis

Using RAPDs, we intended to answer two questions: (1) do different lines differ genetically, i.e., are they different genetical clones; and (2) are worms within a line genetically identical. From each of the lines used in the experiments on genotype–environment interactions, we analyzed three individual worms. Extraction of DNA, polymerase chain reaction (PCR) and electrophoresis followed the protocol by Levitan and Grosberg (1993). Briefly, DNA was extracted with the cetyltrimethyl ammonium bromide (CTAB) method, PCR amplified with random primers (Operon, set C and F) and run on agarose gels. The presence/absence of amplified bands was used to construct a genetic distance matrix based on $1 - S$, where $S = 2N_{AB}/(N_A + N_B)$. N_{AB} is the number of bands shared by individuals A and B, and N_A and N_B are the total number of bands in individuals A and B, respectively. An unrooted tree for relationships among lines was constructed by use of neighbor-joining (Swofford and Olsen 1990) with the neighbor module of PHYLIP 3.5c. (J. Felsenstein, Department of Genetics, University of Washington, Seattle).

High-density cultures

In another experiment we addressed the following questions: (1) Are there differences among lines in carrying capacity? (2) Is there a tradeoff between growth rate in low-density and high-density conditions, i.e., do lines that grow well when worms are cultured individually also grow well when offspring are not removed? (3) If population growth declines at high densities, will individual worms still grow if resources are renewed?

We added three worms from the October cultures from patch A to each of three glass dishes (55 mm inner diameter) with 3 ml of sediment from patch A, and similarly three worms from patch B in three bowls with sediment from patch B. Culture conditions were as described above, but this time worms and sediment were returned to the bowls at each check. The cultures were followed until all worms had died (ca. 75 d). To investigate the ability of worms to regain growth capability, we transferred worms to bowls with new sediment on two occasions (Day 21, before maximum population density was reached and Day 55, after maximum population density), and followed them for 10 d.

Statistical analysis

Analyses of abundance data from the field sampling, and fertility and survival data from culture experiments were made with factorial ANOVA using SYSTAT for Macintosh, Version 5.2 (SYSTAT Inc. Evanston, Illinois, USA). Homogeneity of variances were tested with Cochran's test (Winer et al. 1991), and values were transformed by $\ln(x+1)$ when necessary. Differences among clones in population growth rates were tested with resampling statistics as described in the section "Population matrix analysis." Differences among groups in population growth, mortality and fecundity under high-density conditions were tested with a Kolmogorov–Smirnov two-sample test and with a G-test (Sokal and Rohlf 1981).

Results

Field data

There was a strong seasonal cycle of worm density, both in 1993 and 1994 (Fig. 2). Worms were rare during early spring (and presumably winter), increased in density until June, and decreased again during summer and autumn. There was a significant difference between the two years, with maximum densities in June 1993 higher than in June 1994 (one-way ANOVA: $F_{1,34} = 10.22$, $P = 0.001$). The spatially nested sampling design in June 1993 showed that most of the variation in density (79%) was found between adjacent cores ca. 1 dm apart (Table 1). There was also significant variation among plots within a distance of ca. 5 m. In August and October we found no significant spatial pattern (data not shown). However, the low number of worms found on these occasions (< 10 worms found in 108 samples per date) makes it difficult to conclude anything about the spatial pattern of worms.

Genetic analysis (RAPDs)

Ten primers gave 85 repeatable well-amplified bands (Operon primers C3, C4, C6, C7, C8, C19, C20, F6, F8, F11), as judged by comparison of replicate extractions from the same clone (Fig. 3a). The results show that the five lines used in the genotype–environment experiment have distinct and different banding patterns, i.e., are five different clones (Fig. 3b). Replicate worms within lines shared identical banding patterns. Clones L1 and L2 (collected in March) are genetically most similar to each other, and to a lesser degree resemble clone F2 (collected in May). Clones F1 and F3 (collected in May) are more distantly related to each other, and to the other three clones (Fig. 3b).

Table 1 *Paranais litoralis*. Effect of spatial scale on the number of worms per core. Sites were 30 to 40 m apart, plots (within sites) 5 m apart, patches (within plots) were 1 m apart, and cores (within plots) 1 dm apart

Source of variation	*df*	MS	*F*	*P*	Variance component
Site	2	149.8	0.88	0.46	0[a]
Plot (Site)	6	167.1	3.59	0.016	10.1
Patch (Plot)	18	46.5	1.14	0.33	1.4
Cores (= Residual)	81	40.9			40.9

[a] Calculated variance component actually negative

Differences in growth rate among patches within dates and among dates

There were significant differences in growth rate (expressed as λ per 4 d) among lines in both seasons (Fig. 4) [one-tailed randomization test: $P = 0.023$ (June); $P = 0.021$ (October)]. There were also significant differences in number of offspring produced [one-way ANOVA: $F_{5,54} = 6.44$, $P = 0.0001$ (June); $F_{5,103} = 2.16$, $P = 0.029$ (October)], and in average lifespan for June (one-way ANOVA: $F_{5,54} = 5.67$, $P = 0.0003$) but not for October ($F_{5,103} = 0.916$, $P = 0.473$). Overall, the growth rates in June were significantly higher than growth rates in October (one-way ANOVA: $F_{1,10} = 11.4$, $P = 0.007$), but neither number of offspring produced nor average lifespan differed significantly between seasons (Table 2). The patches supporting the highest growth differed between seasons: the ranking in growth rates in October are almost the reverse of the results from June (Fig. 4). Based on the worm density at the time of sampling in June, we expected patch B to support a low growth rate, and patch C to support a high growth rate. However, there was no significant difference in the growth rate of worms in these patches, either in June or October [pairwise randomization test: $P = 0.45$ (June); $P = 0.38$ (October)].

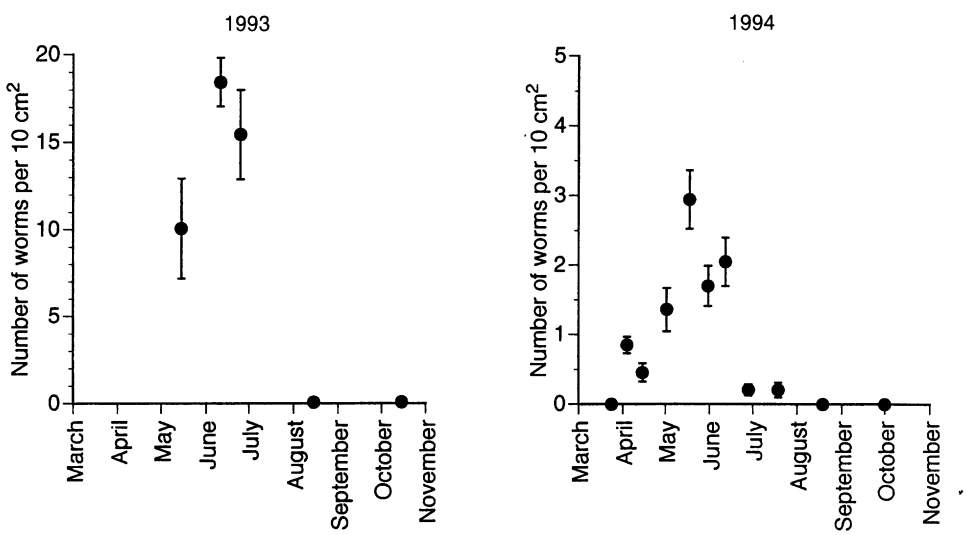


Fig. 2 *Paranais litoralis*. Results from field sampling in 1993 and in 1994. Mean number of worms (±SE) found per 10 cm^2 ($n = 18$)

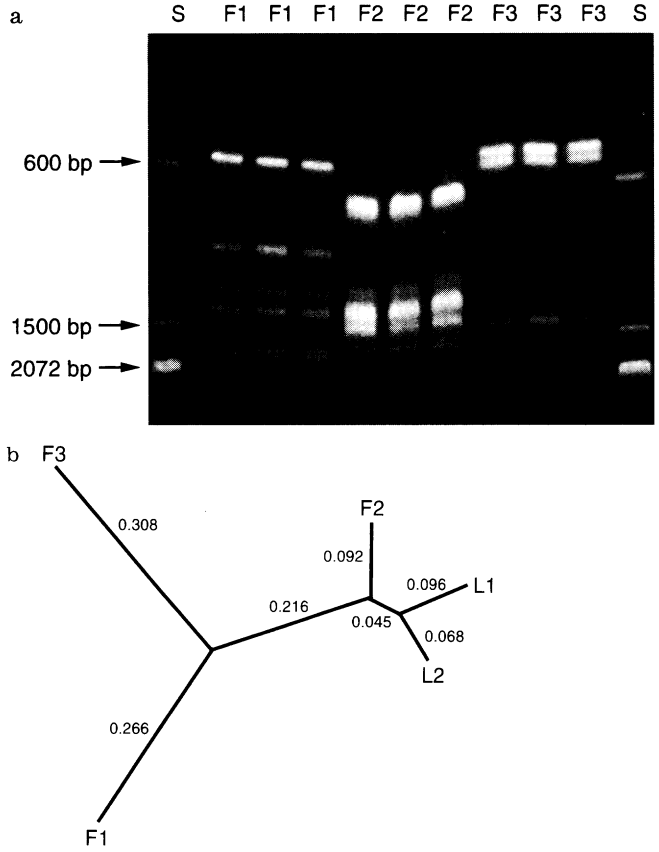


Fig. 3 **a** *Paranais litoralis*. Ethidiumbromide-stained agarose gel showing similarity of banding pattern among worms within a clone, and difference among clones. Three individual worms from each of three lines (*F1*, *F2* and *F3*), amplified with the C6 primer. Lanes labeled *S* show size-markers. **b** Genetic relatedness of the five clones used in experiments for parental effects and genotype–environment interactions, based on RAPD analysis. Unrooted tree constructed by neighbor-joining. Numbers along branches are genetic distances

We found no statistically significant differences in growth rates between worms cultured on their parental sediment or worms cultured in sediment from "good" patches (B and C in June) or "poor" patches (B and C in October). Although growth rates, number of offspring and age of reproduction all indicate that worms cultured on sediment from B or C (e.g., treatments AC, EB and FC in June, see Fig. 4) are intermediate between results on "native" sediment (treatments AA, EE and FF in Fig. 4) and worms from "other" sediment (treatments BB and CC), no differences were significant (one-way or two-way ANOVA, results not shown).

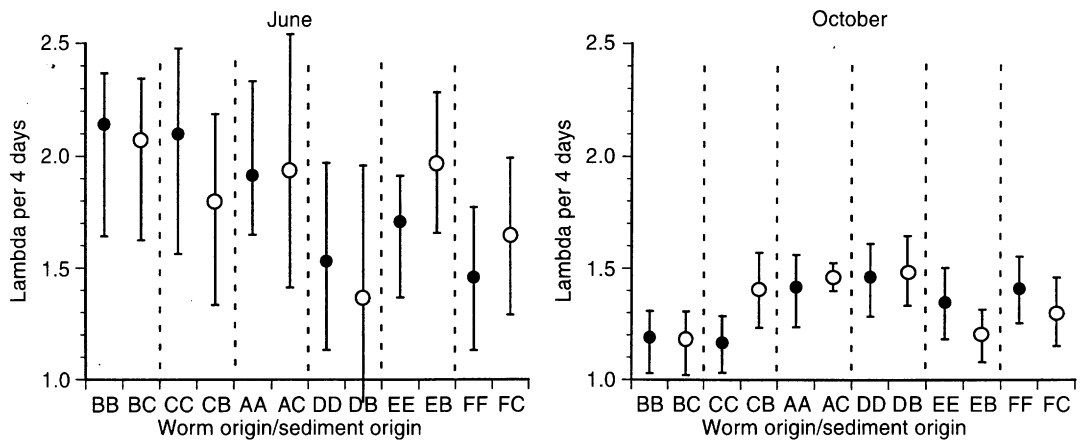
Table 2 *Paranais litoralis*. Effect of season (fixed factor) and patch (random factor), on the lifespan and number of offspring produced by worms in laboratory cultures

Source of variation	*df*	MS	*F*	*P*
Lifespan				
Season	1	801.1	0.49	0.514
Patch	5	1201.6	2.31	0.046
Season × Patch	5	1623.6	3.12	0.010
Residual	157	520.1		
Number of offspring				
Season	1	1063.6	3.35	0.126
Patch	5	198.7	5.47	0.0001
Season × Patch	5	317.1	8.73	0.0001
Residual	157	36.3		

Elasticity

Elasticity analysis of the data from cultures of worms from different patches showed that survival and fertility beyond 30 d of age contributed to $< 1\%$ of growth in June and $< 5\%$ in October, and therefore contribute only trivial differences to λ (Fig. 5). The difference in growth between seasons is due mainly to the number of offspring produced, not survival (Fig. 6a, b), where positive values indicate higher values for June cohorts, and negative values indicate higher values for October cohorts. Worms in June produced more offspring than worms in October, until an age of ca. 60 d (Fig. 6a). The survival was higher for worms from October, mainly late in life (Fig. 6b). However, the contributions of different

Fig. 4 *Paranais litoralis*. Growth rate in laboratory cultures, expressed as growth rate (λ) per 4 d, when cultured on native sediment (marked BB, CC, AA, DD, EE and FF) and on sediment from either patch B or C (marked BC, CB, AC, DB, EB and FC). Data points show bootstrapped means and 95% confidence intervals



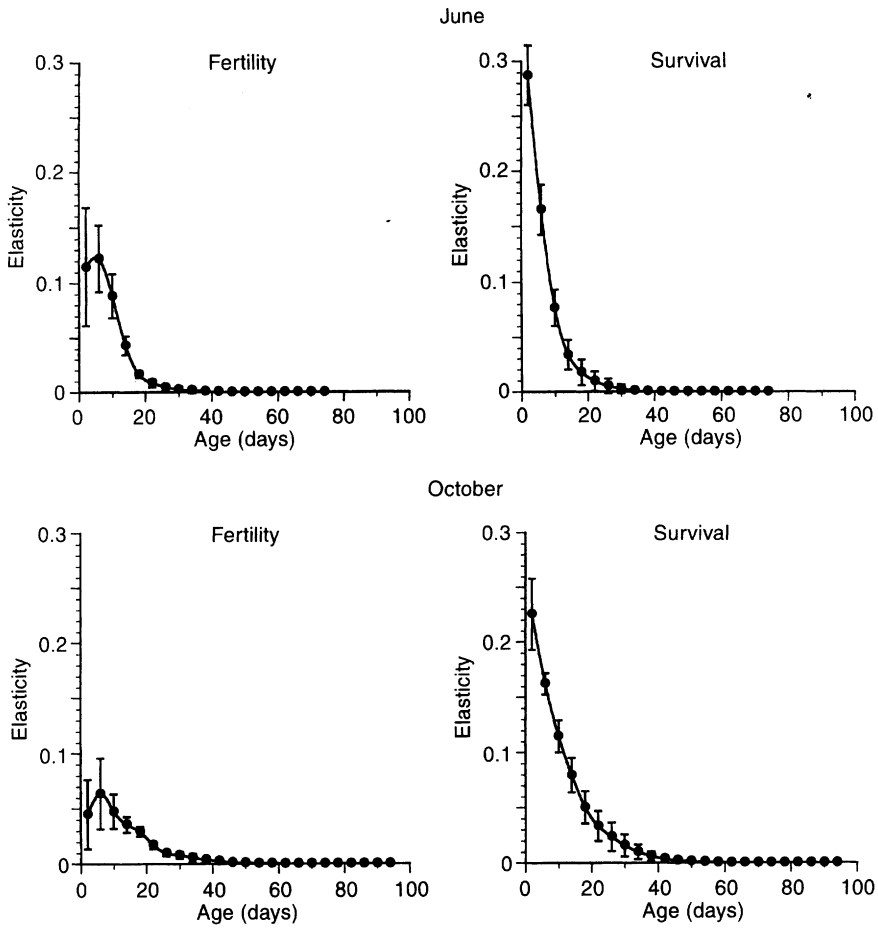


Fig. 5 *Paranais litoralis*. Elasticities of projection matrix elements, averaged over all cultures (A–F) in June (*top two graphs*) and October (*bottom two graphs*). Data are means ± SD ($n = 6$)

elements to differences in growth rate (Fig. 6c, d) is strongly dominated by early reproduction. Elasticities in both June and October were higher for survival than for fertility (Fig. 5), which means that cohorts are potentially more sensitive to changes in the survival rate during early life, but Fig. 6 indicates that little such variation actually contributed to seasonal differences in λ.

Genotype–environment interactions and parental effects

Clones of *Paranais litoralis* differed significantly in fecundity and age at first reproduction (Table 3; Figs. 7, 8). There is no overall parental effect for either fecundity or age at first reproduction ("Parental environment" effect in Table 3), but there is an overall effect of nutritional environment on both dependent variables ("Offspring environment" effect). In general, *P. litoralis* individuals produce more offspring in high- than in low-quality sediment and reproduce at an earlier age in high-quality sediment (Fig. 7). There is also significant interaction between clone and offspring environment for fecundity but not age at first reproduction (Table 3; Fig. 7). This means that lines differ in responses to the nutritional environment experienced by the offspring generation, a genotype–environment interaction. There are also significant interactions between clone and parental environment for both fecundity and age at maturation (Table 3; Fig. 8). This means that some clones display parental effects while others do not. The most dramatic parental effect is for clone L1, which has the highest fecundity of all clones when the parent is cultured under the high nutritional environment, but the lowest rank when the parent is cultured under the low nutritional environment (Fig. 8).

There is a significant three-way interaction in number of offspring produced ("Clone × Offspring environment × Parental environment"; Table 3). The biological significance of this interaction is less clear: its inclusion here is an effect of the experimental setup. However, formally it warns us that any tests including a subset of these three factors (i.e., the parental and genotype-interaction effects discussed above) should be viewed with some caution.

High-density cultures

The cultures went through a rapid phase of growth followed by declining numbers, as we have observed in previous experiments (Levinton and Stewart 1988; Cheng et al. 1993). There was little difference in growth pattern between worms from patch A and worms from patch B (Fig. 9), with no significant difference in maxi-

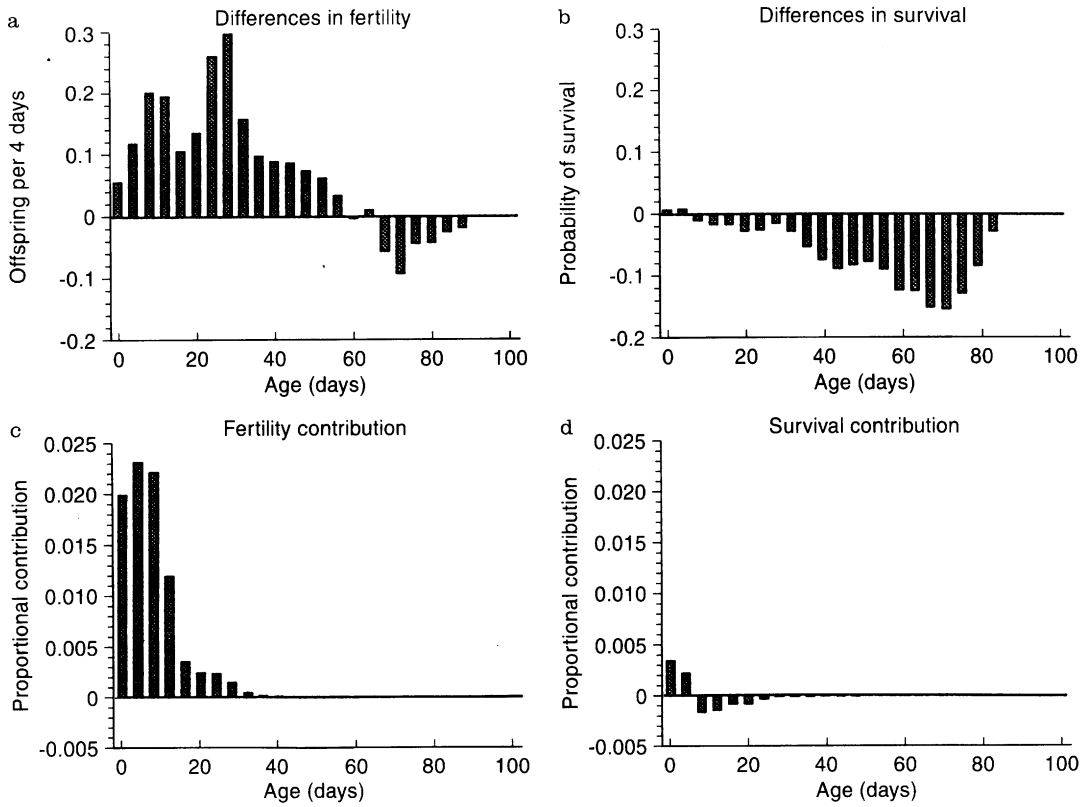


Fig. 6a–d *Paranais litoralis*. Differences between elements of average matrices of June and October in fertility (**a**) and survival (**b**), and contributions of these to differences in growth rate (**c** and **d**)

mum abundance (one-way ANOVA: $F_{1,4} = 0.461$, $P = 0.534$). We could not find any evidence for a trade-off between rapid growth at low densities and high densities. There is a tendency for patch A cultures to grow slightly faster than patch B cultures, as would be expected if the difference in growth rate in low density cultures (Fig. 4) would translate to high density cultures, but this difference is not statistically significant (Kolmogorov–Smirnov two-sample test: $P = 0.43$). Our cultures also confirmed that when *Paranais litoralis* populations started to crash (as after Day 40 in Fig. 9), the worms eventually reached a stage when they no longer could reproduce. Both survival and reproduction were significantly lower after the crash than before the crash (Table 4). Only 2 of the 18 worms reared after the crash reproduced (those two offspring died within a few days), and almost half of the worms died within 10 d.

Table 3 *Paranais litoralis*. Number of offspring and age at first reproduction in genotype–environment experiment. ANOVA: effect of worm clone (random factor), parental environment and offspring environment (fixed factors), and tray effect (random factor nested within each treatment combination)

Source of variation	*df*	MS	*F*	*P*
Number of offspring				
Clone	4	3.69	27.35	< 0.001
Parental environment	1	28.12	4.32	0.107
Offspring environment	1	117.1	63.87	0.013
Clone × Parental	4	6.51	48.24	< 0.001
Clone × Offspring	4	1.83	13.57	< 0.001
Offspring × Parental	1	1.44	0.13	0.740
Clone × Offspring × Parental	4	11.48	85.06	< 0.001
Tray (C × O × P)	20	0.13	0.29	0.998
Individuals (= Residual)	160	0.77		
Age at first reproduction				
Clone	4	88.75	8.16	< 0.001
Parental environment	1	47.05	0.44	0.541
Offspring environment	1	574.6	24.79	0.008
Clone × Parental	4	105.9	9.74	< 0.001
Clone × Offspring	4	23.18	2.13	0.114
Offspring × Parental	1	4.84	0.20	0.677
Clone × Offspring × Parental	4	23.88	2.19	0.106
Tray (C × O × P)	20	10.87	1.63	0.051
Individuals (= Residual)	160	6.90		

Discussion

Asexual lines of *Paranais litoralis* clearly differ from each other in important life-history traits, such as life-span and fecundity. Some of this variation is probably genetic; clones differed significantly also when grown under common conditions during their entire life cycle. The nutritional environment of course affects these life-history parameters: individuals have higher fecundity and earlier maturation in high-quality environments. Of more interest is that some clones also display significant parental effects and genotype–environment interactions.

We isolated a number of worm-lines, all asexually produced offspring from individual field-collected

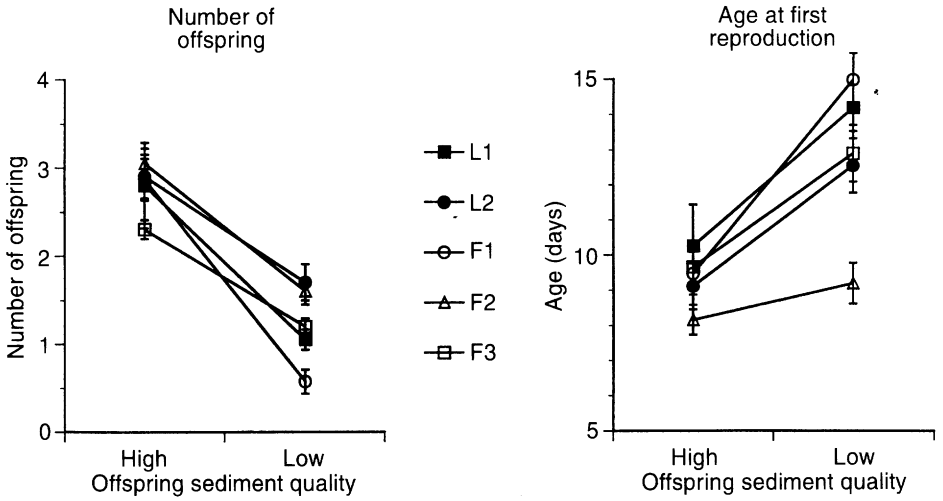


Fig. 7 *Paranais litoralis*. Genotype–environment interactions. Effect of sediment quality experienced by filial generation on number of offspring produced and age at first reproduction. Data are means ± SE (n = 4)

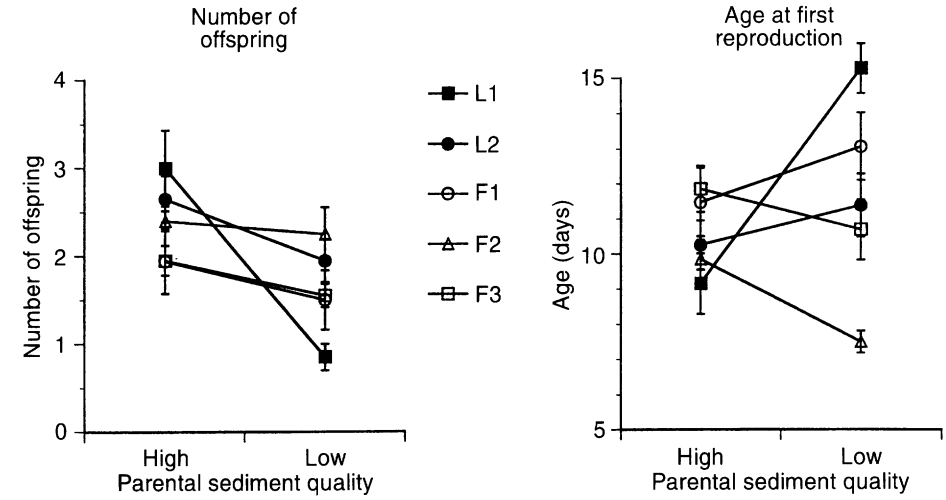
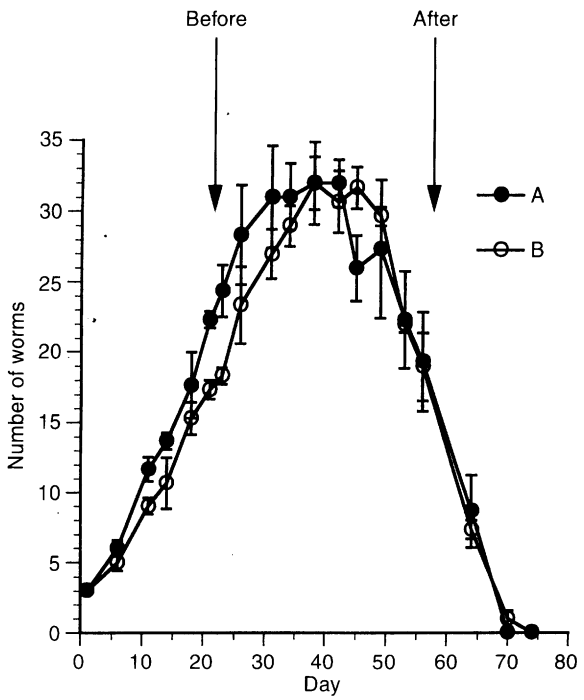


Fig. 8 *Paranais litoralis*. Parental effects. Effect of sediment quality experienced by parental generation on number of offspring produced by filial generation and age at first reproduction of filial generation. Data are means ± SE (n = 4)



worms. All lines used in our genotype–environment experiment appear to be distinct genotypes. RAPDs reliably identified replicate individuals from each line (replicate worms have identical banding patterns). While the different lines used in our experiments appear to belong to different clones, the genetic identity within lines is more problematic, as it is possible that differences exist among lines that we did not find with our set of ten primers (i.e., committing a statistical type 2 error stating that there is no difference when such a difference exists; see also the review by Grosberg et al. 1996). What we can say is that the rate of somatic mutation is so low that we cannot detect genetic differentiation within clones over several generations of asexual reproduction, based on the use of ten RAPD primers.

The genetical analysis indicates that several clones co-occur, and all five clones we analyzed were different.

Fig. 9 *Paranais litoralis*. Number of worms found in high-density cultures started with worms taken from patch A and patch B. Data are means ± SE (n = 3). *Arrows* indicate when worms were taken for analysis of survival and reproduction (see Table 4)

Table 4 *Paranais litoralis*. Survival and reproductive output of worms isolated before or after a population crash (see Fig. 9). Results from *G*-tests on number of survivors and number of reproducing worms after 10 d in "good" sediment

	Before	After
Surviving	20	9
Dead	4	9
$G = 5.16$, $P < 0.01$		
Reproducing	14	2
Nonreproducing	10	16
$G = 10.26$, $P < 0.01$		

What is the origin of the different clones? We can think of at least three possibilities: (1) sexual reproduction has never occurred in Flax Pond, but dispersal of clones from neighboring sexual populations maintains clonal diversity; (2) sexual reproduction did occur and has been lost in Flax Pond, but the loss of genotypes is slow (e.g. due to genotype–environment interactions); or (3) sexual reproduction takes place in Flax Pond but is so rare that we missed it in our field sampling. Pfannkuche (1979) reports that up to 20% of *Paranais litoralis* may be sexually mature in June in a site in the Baltic Sea. However, sexually mature worms have not been found in earlier investigations (spanning almost 18 years, including laboratory and field studies) in Flax Pond (Levinton and Stewart 1988; Cheng et al. 1993; Martinez 1993). If sexual reproduction occurs in Flax Pond, it must be much less common than in the Baltic site. The use of molecular markers can possibly distinguish among the three possibilities mentioned above; if only the same few clones are found throughout the seasonal cycle, then one can potentially rule out the dispersal of clones from sexual populations or sexual reproduction in Flax Pond. If sexual reproduction is taking place, then one would expect to see a high diversity of rare genotypes at least briefly during the season. This will, however, require the sampling and screening of many individuals during a yearly cycle.

If several clones co-occur, and there are significant differences in growth rates, why does not one clone monopolize the mudflat? This could be prevented or slowed down if there are genotype–environment interactions leading to tradeoffs in performance in different seasons and trophic conditions. The clone with the highest average fitness could outcompete the others if the mudflat was an entirely stable and predictable system, but this is probably not the case. Which of the clones that has the largest average fitness will depend on the distribution of sediment quality (whatever the quality is that causes differential growth) and other environmental conditions, and this will vary from season to season, and from year to year. For example, clone L1 (collected in early spring) is a "good colonist"; it performs best under conditions of high resource availability (Figs. 7, 8), as in our stock cultures in the laboratory. Some of the field lines (e.g., clone F2) do well under more limiting resource conditions. Clones L1, L2 and F2 appear to be more genetically similar to each other than to clones F1 and F3 (Fig. 3b). These three clones (L1, L2 and F2) were also the lines that performed best under good conditions, both in terms of number of offspring produced and age at first reproduction (Figs. 7, 8).

We also found significant parental effects, which is to be expected in an asexually reproducing organism such as the populations of *Paranais litoralis* we studied, where the offspring is formed by budding at the tail end of the parent. The biovolume of a newly budded offspring may be one-third of the entire parent's body (Nilsson personal observation), so the investment made by the parent is considerable. However, the parental effects differ among clones (Fig. 8; Table 3): we find parental effects in some clones, but not in others. Parental effects could also increase a source and sink structure: not only would source populations send out more migrating individuals, but the per capita reproduction of the source migrators will also be higher than the per capita reproduction of sink migrators.

The large variation in growth rate among different clones and different sediment qualities we found in our laboratory cultures may, at least partly, explain the large difference in number of worms among patches we found in our field samples (Table 1). There were two levels of spatial resolution that varied the most: between cores 1 dm apart, and between plots 5 m apart. The small-scale patchiness in worm abundance may also be caused by active choice: we have shown that *Paranais litoralis* has the ability to choose high-quality sediment over nutritionally depleted sediment (Nilsson, Levinton and Kurdziel unpublished). Migration may be active (i.e., worms leave a patch when conditions get unfavourable) or passive (e.g., through resuspension of sediment) (Palmer et al. 1996). Our present sampling does not answer at what scale different clones occur (this would be an interesting but much larger investigation), so how these spatial scales relate to active and passive choice is uncertain. The chemosensory ability of worms would counteract genetic division on small scales if all clones had the same preferences, but would promote genetic diversity if clones had different preferences. Another factor causing patchiness may be the pattern of population crashes shown in laboratory cultures: *P. litoralis* populations do not remain at a steady level set by density-dependent regulation, rather the populations are either in rapid growth, or they are rapidly decreasing through mortality or migration.

The elasticity of an element in a population projection matrix measures the relative effect that a change of that particular element would have on the finite rate of increase (Caswell 1989). As long as the population is growing at the rate defined by the particular projection matrix, elasticities can also be seen as the "contribution" of different elements to fitness: a small change in an element with a high elasticity would lead to a relatively large change in fitness (provided that all other elements remain the same; Caswell 1989, p 134). Our results suggest that more than 90% of the "contribution" to

growth rate is during the first 20 d (Fig. 5), the period used in the cultures to investigate parental effects and genotype–environment interactions. What happens thereafter to a particular individual (or unit in clonal organisms) is of little importance both for the fitness of that individual and for the population growth. This holds as long as the population is growing at a fixed rate, described by the projection matrix.

We found that growth rate was positive in all our cultures, both in June and October. Still, in the field overall population level declined. What may cause this discrepancy? Levin and coworkers (Levin et al. 1987; Levin and Huggett 1990) found higher growth rates in laboratory cultures than in field populations of the intertidal polychaete *Streblospio benedicti*, and suggested that differences may be caused by differences in e.g., predation, temperature range, and time available for feeding during the tidal cycle. Here we discuss three factors: predation, competition and physical stress.

In our experiments, worms were not exposed to predation. Oligochaetes are eaten by a wide variety of organisms, including wading birds, juvenile flatfish, crustaceans, polychaetes, nemerteans and turbellarians (Giere 1993). However, in field experiments using predator exclusion cages in Flax Pond, Cheng et al. (1993) found significant predation effects on other endobenthic taxa (e.g., *Streblospio benedicti*), but no effects were observed on *Paranais litoralis*. On the mudflat in Flax Pond, populations of the omnivorous polychaete *Nereis succinea* are very dense during summer and early autumn (Levinton and Nilsson personal observation), and they may negatively affect *P. litoralis* populations through predation, disturbance or competition. In the laboratory cultures, interference competition with *N. succinea* was excluded. However, we should have detected a decline due to exploitative competition, because we fed the worms sediment collected anew from Flax Pond each week, so we do not think that absence of exploitative competition is a likely explanation for the difference between laboratory growth and the population decline in the field. In the laboratory experiments, we also sheltered the worms from physical extremes in, e.g., temperature and salinity. Previous laboratory experiments have shown that population growth of *P. litoralis* from Flax Pond is slower at 25 °C compared to 18 °C (Levinton and Stewart 1988; Cheng et al. 1993), and temperatures above 25 °C may even be lethal (authors' personal observation). The water temperature in Flax Pond seldom reaches 25 °C, but surface sediment temperatures may exceed this during summer and early autumn (Cheng et al. 1993), causing the worms to move to deeper, cooler sediment. However, the deeper sediment layers are of lower nutritional value at least during periods (Cheng et al. 1993), and may be anoxic which would make it inaccessible to the worms.

We can summarize by going back to the original questions asked in the introduction: (1) Is the population growth rate of *Paranais litoralis* heterogeneous among patches on an intertidal mud flat? Yes, growth experiments show significant differences among worms and sediment collected at different patches. (2) If growth rates are heterogeneous, do some places consistently support higher growth rates than others, or does this change with time? The patches supporting the highest growth differed between seasons. (3) If growth rates are heterogeneous, is this caused by sediment quality, by factors intrinsic to the worms, or by a combination thereof? Are there genotype–environment interactions and/or maternal (parental) effects in this species? Part of the variation in life-history traits among clones is probably genetic, since clones differed significantly when grown under the same conditions during their entire life cycle. The nutritional environment affected these life-history parameters, with individuals cultured in high-quality environments having higher fecundity and earlier maturation. We also found significant genotype–environment interactions and parental effects. (4) Do differences in population growth under low-density conditions remain during high-density conditions? We could not detect any significant differences between clones grown under high-density (resource-depleted) conditions. (5) Can RAPD be used to distinguish among clones? If so, is more than one clone of *P. litoralis* present at the mud flat? Yes, RAPDs could discriminate between several clones collected in Flax Pond.

In conclusion, data from field samples and laboratory cultures suggest that the population growth of *Paranais litoralis* in Flax Pond is both spatially and temporally heterogeneous. This is not an example of a population subdivided into a set of *permanent* source and sink subpopulations, but rather of a continuously shifting mosaic of local growth conditions.

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