

Bone-eating *Osedax* females and their 'harems' of dwarf males are recruited from a common larval pool

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Abstract

Extreme male dwarfism occurs in *Osedax* (Annelida: Siboglinidae), marine worms with sessile females that bore into submerged bones. *Osedax* are hypothesized to use environmental sex determination, in which undifferentiated larvae that settle on bones develop as females, and subsequent larvae that settle on females transform into dwarf males. This study addresses several hypotheses regarding possible recruitment sources for the males: (i) *common larval pool* – males and females are sampled from a common pool of larvae; (ii) *neighbourhood* – males are supplied by a limited number of neighbouring females; and (iii) *arrhenotoky* – males are primarily the sons of host females. *Osedax rubiplumus* were sampled from submerged whalebones located at 1820-m and 2893-m depths in Monterey Bay, California. Immature females typically did not host males, but mature females maintained male 'harems' that grew exponentially in the number of males as female size increased. Allozyme analysis of the females revealed binomial proportions of nuclear genotypes, an indication of random sexual mating. Analysis of mitochondrial DNA sequences from the male harems and their host females allowed us to reject the *arrhenotoky* and *neighbourhood* hypotheses for male recruitment. No significant partitioning of mitochondrial diversity existed between the male and female sexes, or between subsamples of worms collected at different depths or during different years (2002–2007). Mitochondrial sequence diversity was very high in these worms, suggesting that as many as 10^6 females contributed to a common larval pool from which the two sexes were randomly drawn.

Keywords: Annelida, *Osedax rubiplumus*, paedomorphosis, recruitment, Siboglinidae

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Introduction

The males and females of a gonochoric animal are expected to be of equal size unless sexual or natural selection drives them apart (Darwin 1874; Vollrath 1998). The strength of these evolutionary forces can be seen in extreme cases of size dimorphism, one of which is the phenomenon of dwarf males. Animal species with dwarf males have evolved multiple times and are known among annelids, barnacle and copepod crustaceans, bivalve, gastropod and cephalopod molluscs, cycliophorans, rotifers, spiders and fishes (reviewed in Vollrath 1998). Ghiselin noted in his pioneering treatise, *The Economy of Nature & the Evolution of Sex* (1974, p. 193) that dwarf males 'occur most often in smaller organisms,

and in the sea, especially at greater depths. However, the forms that do display the phenomenon are by no means rare, and they occur scattered about in the various branches of the phylogenetic tree.' Furthermore, he noted that the benefits of male dwarfism derive from increased dispersal potential of smaller males and from reduced food competition with females, advantages that should be most pronounced with restricted motility (sedentary or sessile species), or at low population densities. An absence of male–male competition for females at low densities would benefit the smaller and more motile males, which in turn should favour accelerated development, early maturation, or paedomorphosis.

Male dwarfism has evolved to a marked degree in species of *Osedax* (Annelida: Siboglinidae), which recently became known to science (Rouse *et al.* 2004). *Osedax* females are several orders of magnitude larger than the microscopic males that they host in their gelatinous tubes. The sessile

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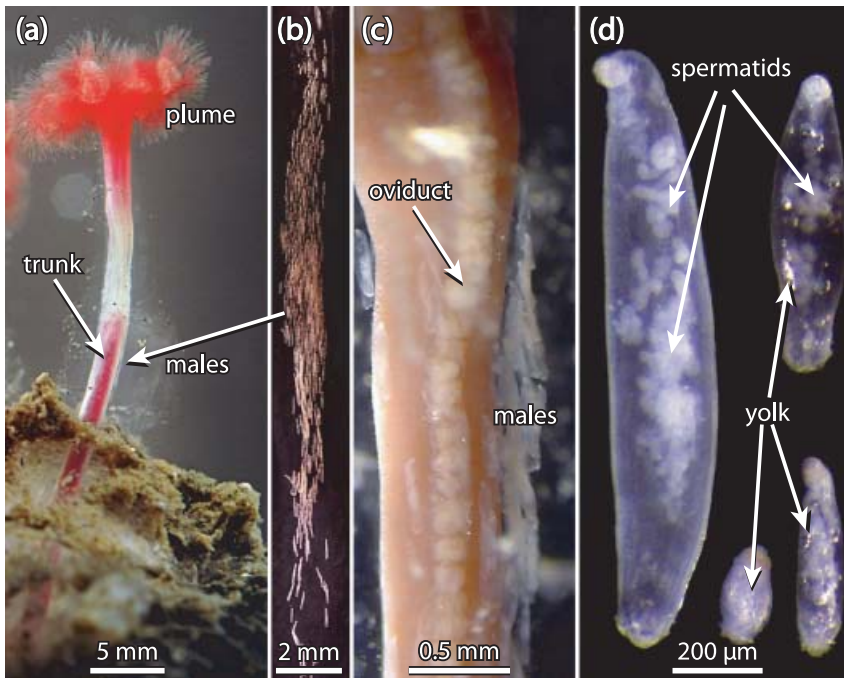


Fig. 1 *Osedax rubiplumus* males and females. (a) Adult female on bone photographed in an aquarium immediately after recovery from ROV Tiburon. The female's plume has contracted from the normal condition *in situ* and has retracted slightly into the transparent tube surrounding the trunk. A harem of microscopic males lies next to her trunk in the lumen of the tube (arrow). (b) A harem of males attached to the transparent tube after removal of the female. (c) The anterior trunk of a female, showing a harem of males lying adjacent to her oviduct. (d) Four males from a single harem, illustrating the extent of size variation among males. The smallest male still is full of yolk, but the mid-sized specimens have optically refringent yolk granules and spermatids. The largest male has no obvious yolk granules.

females lack a digestive system, developing instead a complex 'root' system that bores into bones to extract nutrients with the aid of endosymbiotic bacteria that degrade organic compounds (Goffredi *et al.* 2004, 2007). *Osedax rubiplumus* and *Osedax frankpressi* were the first described species, from a whale carcass found at 2893 m depth in Monterey Bay, California, but additional species are now known from shallower whale-falls around the world: *Osedax mucofloris* at 30–125 m off Sweden (Glover *et al.* 2005); *Osedax japonicus* at 224–250 m off Japan (Fujikura *et al.* 2006); and *Osedax roseus* at 1018–1820 m also in Monterey Bay (Rouse *et al.* 2008). Five additional undescribed species are known from depths ranging between 385 m and 2893 m in Monterey Bay (Braby *et al.* 2007; Jones *et al.* 2008). Dwarf males were initially reported as absent in *O. mucofloris* and *O. japonicus* (Glover *et al.* 2005; Fujikura *et al.* 2006), but they have now been observed in all *Osedax* species examined to date (Rouse *et al.* 2008). Among the siboglinid worms, dwarf males appear to be unique to *Osedax*, as the sexes are of equal size in vestimentiferans and frenulates. *Osedax* males typically occur in 'harems', clusters that reside inside the female's tube immediately adjacent to her trunk (Fig. 1). Ranging from ~0.2–1.0 mm in length, the males are markedly paedomorphic, arresting development at a metatrochophore larval stage (Rouse *et al.* 2004, 2008).

Environmental sex determination (ESD) is hypothesized to operate in *Osedax* (Rouse *et al.* 2004, 2008) as in the echiuran annelid *Bonellia viridis* (Baltzer 1931, 1934; Charnov & Bull 1977; Jaccarini *et al.* 1983). *Bonellia* larvae that settle alone onto the bottom develop into females, but the larvae

subsequently settling on these females are transformed into dwarf males that live within the female's body. Suitable cracks and crevices in or under rocks are a limiting resource for *Bonellia* females, whereas females are a limiting resource for the males. Similarly, *Osedax* females must find bones to colonize and males must find the females. Although scattered, whale carcasses are believed to occur at relatively frequent intervals (5–15 km) along the California margin, a migration route for several large cetacean species (Smith & Baco 2003), but *Osedax* also appear capable of subsisting on smaller mammalian bones (Jones *et al.* 2008; Vrijenhoek *et al.* 2008). Yet even the large bones of a blue whale can degrade over the course of a few years in places like Monterey Bay, California (Braby *et al.* 2007), so rapid growth and early reproduction would confer an advantage to *Osedax* females. Indeed, *Osedax* species are capable of colonizing bones and growing to sexual maturity in as little as 1 month (Glover *et al.* 2005) to 3 months (Rouse *et al.* 2008). *Osedax rubiplumus* females can reach densities of 3–20 individuals per square centimetre, covering exposed bones with their crown of plumes and broad gelatinous tubes, a condition that would limit settlement of late-arriving larvae on bone and favour the recruitment of dwarf males. More than 100 dwarf males could be found in the tubes of mature *O. rubiplumus* females, and male harem sizes appear to increase exponentially with female age (Rouse *et al.* 2004).

The high diversity of mitochondrial DNA (mtDNA) haplotypes found in discrete samples of *O. rubiplumus*, *O. frankpressi* and *O. roseus* indicate that neighbouring females are products of numerous independent propagules that

settled from vast larval pools (Rouse *et al.* 2004, 2008). To date, the dwarf males have not been studied genetically. Here we examine several hypotheses concerning patterns of male recruitment in *O. rubiplumus*. (i) The null hypothesis posits that males and females are recruited equivalently from a *common larval pool*. (ii) The *neighbourhood* hypothesis posits that the males in a harem may be products primarily of local recruitment. We have noted early embryos attached to the gelatinous tubes of females of several *Osedax* species in laboratory aquaria. Females may intercept zygotes produced by their neighbours, swamping the recruitment of males from a common larval pool. Finally, (iii) the *arrhenotoky* hypothesis posits that the males in a harem may be products of parthenogenesis. For example, *O. japonicus* females have short oviducts and have been suggested to retain embryos in their gelatinous tubes (Fujikura *et al.* 2006). Although arrhenotoky involving the production of haploid males is not known among annelids, it has evolved independently in a range of animal taxa including some rotifers, nematodes, and a number of arthropods including some scorpions, beetles, and most notably in the Hymenoptera (Otto & Jarne 2001). Parthenogenetic all-female reproduction has evolved in several clitellate annelid families, which are otherwise always hermaphroditic (reviewed in Suomalainen *et al.* 1987).

We examined two classes of molecular markers to test hypotheses regarding reproduction in *O. rubiplumus*. Bones housing this species were sampled from two whale carcasses at different depths in Monterey Bay, California. Allozymes were examined to determine whether the worms exhibit genotypic frequencies expected for a randomly mating population. Mitochondrial DNA sequences were examined to test the previously listed hypotheses about male recruitment. If *arrhenotoky* exists, a large portion (or all) of the males in a harem should bear the host female's mtDNA. If the *neighbourhood* hypothesis is correct, the males in a harem will not likely carry the host female's mtDNA, but they should exhibit greater co-ancestry than expected for a random sample of individuals, because the harem is likely to contain siblings. Finally, if the *common larval pool* hypothesis is correct, males and females will comprise genetically equivalent subsamples regardless of when (date) or where (depth) the whalebones were deposited.

Materials and methods

Samples

Bones containing *Osedax rubiplumus* were sampled with the Monterey Bay Aquarium Research Institute's remotely operated vehicle (ROV) *Tiburon* from grey whale carcasses in Monterey Bay, California. Details regarding the origin, location and ecological setting of each carcass were previously described (Braby *et al.* 2007). Whale-2893

(coordinates: 36.613°N, 122.434°W) was discovered during routine exploration of the Monterey Canyon floor at 2893 m depth on 6 February 2002 (Goffredi *et al.* 2004). The beached carcass of whale-1820 (36.708°N, 122.105°W) was towed from shore and sunk at 1820 m depth on 20 March 2006. We collected *Osedax*-bearing bones from whale-2893 during *Tiburon* dive T486 (9 October 2002), and from whale-1820 during dives T990 (23 May 2006), T1048 (24 October 2006), and T1071 (12 January 2007). Each of the sampled bones was photographed *in situ*, placed in a thermally insulated 'biobox' on *Tiburon*, and subsequently transferred to cold (4 °C) filtered seawater upon recovery at the surface. A part of each bone containing *Osedax* was preserved in 95% ethanol for molecular analyses, and another portion was preserved for light microscopy in 4% formalin-seawater. One sampled bone from dive T1048 was frozen at -80 °C for allozyme analysis of the worms.

Reproductive characters

Females were dissected from formalin or ethanol-preserved bone and gently removed from their gelatinous tubes while under a Leica MZ8 stereomicroscope. Female crown length and trunk length and width (just posterior to crown) were measured with an ocular micrometer on formalin-preserved specimens. The appearance of eggs in the transparent oviduct was used as an indicator of reproductive maturity and spawning. The tubes of the females were then examined for males, which were counted when found. For a series of harems, the length of the dwarf males were all individually measured. Micrographs of harems or males were taken with Nikon Coolpix 4300 cameras on either a Leica MZ8 stereomicroscope or Leica DMR compound microscope. All statistical analyses used to examine reproductive characters were conducted with JMP version 7.0.1 software (SAS Institute Inc. 2007).

The dwarf males examined for mtDNA haplotypes were removed from host females that had been preserved in 95% ethanol. Each host female also had part of her crown removed for mtDNA analysis. The tube enclosing each female was then carefully removed and examined under a dissecting microscope for the present dwarf males. A subset of approximately 10 males was removed with fine forceps and males were stored individually in 95% ethanol before DNA extraction.

Allozymes

Allozymes were examined in a frozen sample of *O. rubiplumus* females from dive T1048. Plume tissue was excised from individuals and homogenized in a roughly equal volume of extraction buffer (0.01 M Tris, 2.5 mM EDTA, pH 7.0). The homogenate was centrifuged at 8000 × G for 2 min to remove tissue debris. Routine cellulose acetate gel

electrophoresis (CAGE) followed the methods of Hebert & Beaton (1989) to screen 10 enzyme systems (malate dehydrogenase, mannose phosphate isomerase, phosphoglucomutase, aspartate aminotransferase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, phosphoglucose isomerase, and leucyl-glycine and leucyl-glycyl-glycine peptidases). Several enzyme systems were polymorphic, but the multibanded patterns observed for mannose phosphate isomerase and isocitrate dehydrogenase involved simultaneously polymorphic products from more than one locus and could not be resolved. Only the phosphoglucose isomerase patterns were consistently scorable. Males were not examined, as they were very small and could not be removed intact from the frozen tubes. An exact test of Hardy–Weinberg genotypic frequencies was conducted with GenePop version 3.3 (Raymond & Rousset 1995).

Mitochondrial DNA

General methods for purifying and sequencing mitochondrial COI (mtCOI) from *Osedax* were previously described (Rouse *et al.* 2004, 2008). The vestimentiferan-specific primers COIF and COIR (Nelson & Fisher 2000) were used to amplify a 1200-bp fragment of mtCOI. We designed an internal primer, LCO1708 5'-GCYCCAGATATAGCWTTCCC-3', to ensure complete coverage of the fragment. All DNA sequences were obtained bi-directionally, proofread using Sequencher version 4.7 (Gene Codes Corp. Inc.), and edited by eye using MacClade version 4.08 (Maddison & Maddison 2005). The 69 unique sequences, 999 bp in length, obtained in this study were deposited under GenBank Accession nos EU852420–EU852488.

The program DnaSP version 4.10.9 (Rozas *et al.* 2003) was used to estimate: S , the number of segregating (polymorphic) sites; H , the number of haplotypes; h , haplotype diversity (equation 8.4, Nei 1987), π , nucleotide diversity (equation 10.5 in Nei 1987); and θ , per site from S (equation 10.3, Nei 1987). The program Arlequin version 3.1.1.1 (Excoffier *et al.* 2005) was used to conduct AMOVAS and demographic analyses, and TCS version 1.21 (Clement *et al.* 2000) was used to construct statistical parsimony networks.

Results

Reproductive characters

Clusters of dwarf males, harems, were clearly visible inside the lumen of the transparent tube that enclosed most *Osedax rubiplumus* females (Fig. 1a, b). The males typically clustered adjacent to the oviduct (Fig. 1c), which extends along the trunk from the posterior ovary to the anterior plume. Due to their fork-like chaetal hooks, males remained attached to the inner wall of the tube when the female was

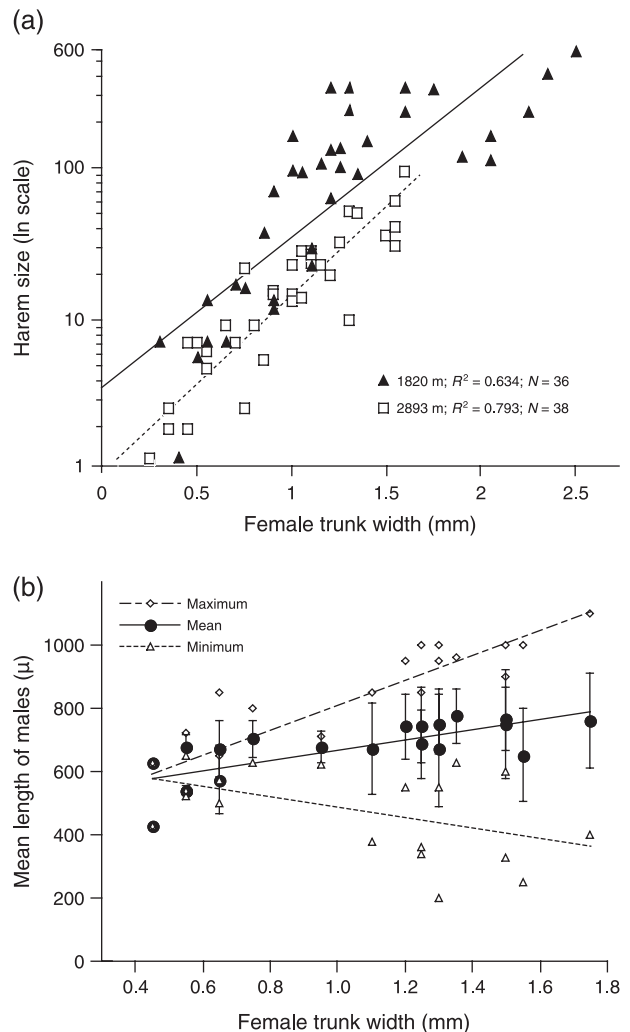


Fig. 2 Variation in male harems associated with females of varying size. (a) Number of males per harem (\log_e scale) plotted against female trunk width from samples taken from whale-1820 (triangles) and whale-2893 (squares). (b) Length distributions of males from 20 individual harems (a subset of the sample analysed from whale-2893 in Fig. 2a) shown as means (closed circles) \pm 1 SD, maximum and minimum length of males plotted against female width.

removed (Fig. 1b). The average length of 397 males sampled from 20 separate females (Fig. 2b), was 719.6 μm (range: 200–1000 μm). The males have no mouth or digestive tract (Fig. 1d) and appear to subsist solely on stored yolk that is retained from the egg stage. Various stages of spermiogenesis were evident in males (Fig. 1d). Smaller males were densely packed with yolk granules and had relatively few spermatid bundles. Larger males contained less yolk and except for bundles of spermatids appeared to be mostly empty.

Size variation was examined among 95 females sampled from the two whale carcasses (Table 1). The subsample from whale-1820 contained larger females on average than the subsample from whale-2893. For the combined sample of 95

Table 1 Sizes of *Osedax rubiplumus* females in contrasting subsamples (measured as trunk width in millimetres)

Contrast	Mean A	<i>n</i>	Mean B	<i>n</i>	<i>F</i>	<i>P</i>
(A) Whale-1820 vs. (B) whale-2893	1.20 ± 0.61	41	0.92 ± 0.47	54	3.40	< 0.001
(A) Mature vs. (B) immature	1.23 ± 0.48	61	0.82 ± 0.62	34	12.63	< 0.001
(A) With vs. (B) without a harem	1.27 ± 0.50	74	0.42 ± 0.01	21	11.18	< 0.001

n = sample size; *F* = *F*-value from analysis of variance; *P* = probability.

females, spawning individuals (defined as having eggs in the oviduct) were larger on average than immature females. Altogether, 74 females hosted one or more males (57 were spawning and 17 had no obvious eggs). These 74 females were three times as wide as the females that hosted no males.

Male harem sizes were not normally distributed; however, \log_e -transformed counts of the males were normally distributed (Shapiro–Wilk $W = 0.974$; $P = 0.220$) among the 57 mature females, which hosted a geometric mean of 26.2 male each. In addition, 17 not obviously spawning females hosted smaller harems with a geometric mean number of 4.9 males, but these \log_e -transformed counts were not normally distributed ($W = 0.862$; $P < 0.017$). The transformed harem sizes associated with these 74 females were tightly correlated with female trunk width (Fig. 2a). Trunk and crown lengths were similarly correlated with harem size, but these longitudinal measurements were highly variable in the retractile worms and explained less of the covariance with harem size. The maximum number of males observed in a harem was 607 hosted by a single female from whale-1820 vs. a maximum of 111 males hosted by a single female from whale-2893. After accounting for size differences between females sampled from the two whales, harem sizes still tended to be larger in the whale-1820 subsample (Fig. 2a).

Osedax rubiplumus females appeared to recruit new males throughout their reproductive lifespans. Male size-frequency distributions within individual harems increased dramatically in breadth as the host females increased in size, a pattern that was most evident in plots of the minimum and maximum sizes of the males (Fig. 2b). The standard deviation of male length was strongly correlated with female size ($r = 0.79$, $P < 0.001$, $n = 20$).

Allozymes

We examined allozyme patterns to assess whether *O. rubiplumus* might participate in random sexual mating. Heterozygous individuals for the *Pgi* locus exhibited three bands: a fast dimer (FF), a heterodimer (FS), and a slow dimer (SS) in roughly 1:2:1 proportions. Inferred genotypes (*ff*, *fs*, *ss*) occurred in the following proportions: 8, 15, 6. Binomial expectations were 8.3, 14.4, 6.3 and $\chi^2 < 0.001$ (d.f. = 1; $P = 0.078$). No evidence was found for a deviation from random-mating expectations. Examination of additional

Table 2 Mitochondrial COI sequence diversity in male and female *Osedax rubiplumus* from Monterey Bay, California

Statistic	Females	Males	Total
Sample size <i>N</i>	77	116	193
No. of haplotypes <i>H</i>	35	47	69
Haplotype diversity <i>h</i>	0.912	0.936	0.926
Segregating sites <i>S</i>	50	52	74
Nucleotide diversity π	0.00312	0.00279	0.00292
Theta per site θ	0.01018	0.00977	0.01269
Standard deviation of θ	0.00292	0.00264	0.00308

polymorphic nuclear loci would be desirable, but other enzyme loci were not adequately resolved with the CAGE methods applied to this species.

Sequence diversity

Sequence analysis of 999 bp of mitochondrial COI sequences revealed a high level of nucleotide diversity (Table 2). We observed 69 unique haplotypes that varied at 74 sites in this sample of 193 *O. rubiplumus* (77 females and 116 males). All nucleotide substitutions were synonymous. Three haplotypes (labelled *a*, *b* and *c* in Fig. 3a) together comprised 42% of the total sample. Haplotype *b* was inferred as the oldest haplotype based on the methods and assumptions implemented by the rcs program. Males and females were essentially identical with respect to haplotype diversity *h*, the number of segregating sites *S*, nucleotide diversity π , and theta per site θ (Table 2).

Tests of male recruitment hypotheses

A maximum parsimony network, constructed to visualize relationships among the 69 COI sequences, revealed no apparent clustering of haplotypes between the two sexes (Fig. 3a). Furthermore, no statistically significant component of variance existed between the male and female subsamples ($F_{ST} = 0.0013$; $P = 0.4050$). Disregarding sex, hierarchical AMOVA revealed no significant variance components due to subsamples taken at different depths, 2893 vs. 1820 m ($F_{CT} = 0.0240$; $P = 0.1486$) or on different dive dates nested within the two depths ($F_{SC} = 0.0165$; $P = 0.1574$). We conclude therefore, that all the subsamples of these worms,

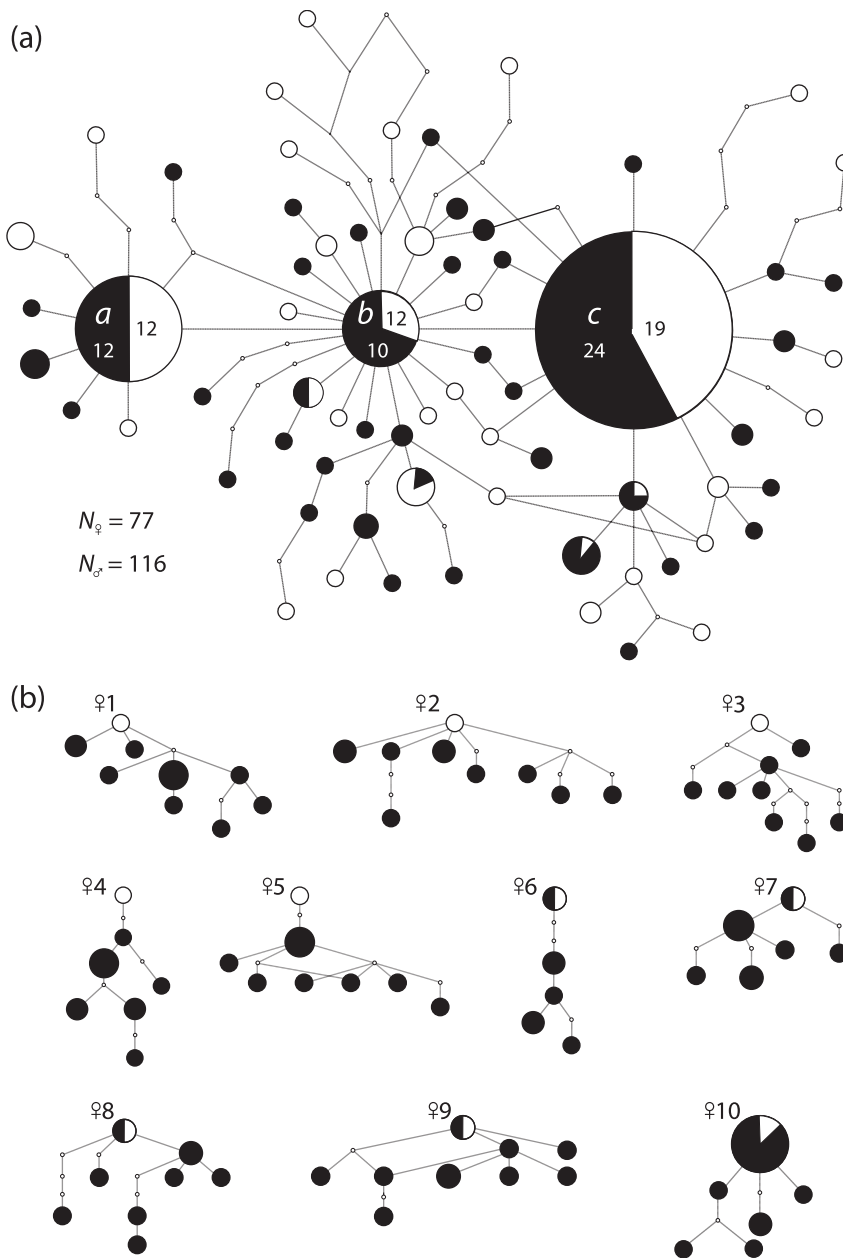


Fig. 3 Mitochondrial haplotype networks. (a) Total network of male (black) and female (white) haplotypes from sample of 193 *Osedax rubiplumus*. Circles are proportional to their frequency, and sample sizes are given for the most frequent haplotypes (*a*, *b* and *c*). Missing haplotypes are indicated with small open circles. (b) Haplotype networks for 10 females (circles labelled 1 through 10) and associated males.

regardless of partitioning by sex, depth or date, were drawn from the same 'mitochondrial' pool.

We also constructed maximum parsimony networks to assess relationships between the males in a harem and their host-female (Fig. 3b). Only harems for which we obtained at least seven male sequences were considered in this analysis. The *arrhenotoky* hypothesis could be rejected unequivocally for five of the 10 harems that we examined (females 1–5), as none of these males shared their host-female's mitochondrial haplotype. For the remaining five harems (females 6–10), one or more males in the harem shared the host-female's haplotype. If we assume potential males were drawn at random from a larval pool, their haplotypes would be

independent of the female's haplotype. Thus, if q_i is the expectation of a host-female's haplotype in the total population, the probability of obtaining n males sharing haplotype i in a sample of N males is defined by binomial equation:

$$\Pr(n) = \frac{N!}{n!(N-n)!} q_i^n (1-q_i)^{N-n}$$

The probabilities of shared male haplotypes for females 6–10 indicate that this degree of sharing is likely due to chance alone (Table 3). Consequently, we could not reject the null hypothesis of independent draws of these males from a *common larval pool*. We can also use this method to

Table 3 Binomial expectations, $Pr(n)$, for obtaining n males in a sample of N males that share a mitochondrial haplotype occurring at frequency q_i with the host female

Female	n	N	q_i	$Pr(n)$
1	0	11	0.124	0.232
2	0	10	0.223	0.080
3	0	8	0.026	0.810
4	0	10	0.026	0.769
5	0	9	0.005	0.954
6	1	7	0.026	0.155
7	1	9	0.124	0.387
8	1	9	0.223	0.267
9	1	10	0.223	0.230
10	4	10	0.223	0.114

determine the probability that females 1–5 would host a harem that did not include one or more males with a shared haplotype. Again, the probabilities are high (Table 3), and we cannot reject the *common larval pool* hypothesis.

Finally, we tested the *neighbourhood* hypothesis by assessing whether the males in a harem were more closely related to one another (coming from a limited number of neighbours) than expected by chance. The total sample of males was partitioned according to female host. No evidence for co-ancestry was found among male harems ($F_{ST} = 0.0111$; $P = 0.5011$). Together, all analyses of the mitochondrial data were consistent with the hypothesis that these males and females of *O. rubiplumus* were recruited in an unbiased manner from a *common larval pool* that did not vary significantly according to bathymetric depth or on the timescale of this study.

Demographic analysis

The effective number of breeding females [$N_{e(f)}$] contributing to this larval pool was estimated from levels of synonymous variation in mitochondrial COI, according to the relationship $\theta = 2N_{e(f)}\mu$. Mutation rate, μ , was previously estimated to be $\mu_1 \approx 3 \times 10^{-9}$ substitutions per site per generation (for methods see Rouse *et al.* 2004). Given the obtained value of $\theta = 0.01320$ (Table 2), we estimated that $N_{e(f)} = \theta / (2\mu_1) \approx 2.2 \times 10^6$. However, our value $\mu_1 \approx 3 \times 10^{-9}$ might be too slow by an order of magnitude for a within-species COI mutation rate (e.g. Audzijonyte & Väinölä 2006). Substituting a faster rate, $\mu_2 = 3 \times 10^{-8}$, correspondingly reduced our estimate of effective population size, $N_{e(f)} = \theta / (2\mu) \approx 2.2 \times 10^5$. Despite our inadequate estimates for μ , we must still conclude that a large number of *O. rubiplumus* females contributed to the larval pool that colonized these Monterey whale carcasses.

Tajima’s D was markedly negative in the total sample (-2.389 ; $P < 0.01$) and the distribution of mismatches for

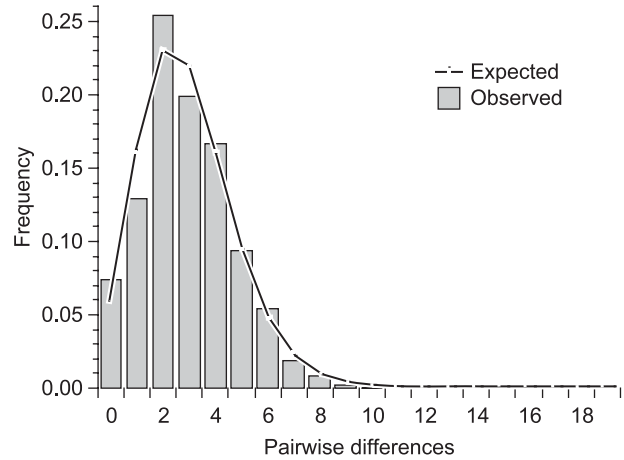


Fig. 4 Observed mismatch distribution (vertical bars), and the frequency distribution expected for an expanding population, assuming coalescent time $\tau = 3.008$, $\theta_0 = 0$, $\theta_1 = 52.7$.

these 193 COI sequences (Fig. 4) was smooth and unimodal (raggedness statistic $r = 0.0309$; $SSD = 0.00221$; $P = 0.250$). To assess the possibility that this population does not meet expectations for size stationarity, we used the nonlinear least-square approach (Schneider & Excoffier 1999) as implemented in Arlequin 3.1 (Excoffier *et al.* 2005) to estimate demographic parameters. We estimated t , the age of this demographic expansion from the relationship $\tau = tv$, where $v = \mu L$ is the mutation rate per gene of length $L = 999$ bp. We used the estimated coalescent time $\tau = 3.008$ and let $\theta_0 = 0$, $\theta_1 = 52.7$. We also let v range from a fast rate ($\mu_2 L = 3 \times 10^{-5}$) to a slower rate ($\mu_1 L = 3 \times 10^{-6}$) and obtained estimates of t that ranged from 10^5 to 10^6 generations.

Discussion

Our results are consistent with the hypothesis that males and females of *Osedax rubiplumus* living on submerged whalebones in Monterey Bay, California, are recruited from a common larval pool. Results from a single nuclear allozyme locus (*Pgi*) were consistent with the hypothesis that *Osedax* females are products of random sexual mating, but this observation alone does not allow us to exclude the *arrhenotoky* or the *neighbour* hypotheses for males. Nonetheless, the males in harems typically did not share their host female’s mitochondrial haplotype, as would be expected if *arrhenotoky* produced most of the males. Additionally, the male harems exhibited no evidence for co-ancestry, as expected if produced by a limited number of neighbouring females. Normalized sequence diversity for the mitochondrial COI haplotypes found in males and females of *O. rubiplumus* was high ($\theta = 0.01269$). Consequently, our estimate of the effective number of females contributing to this population also was large, $N_{e(f)} = 2.2 \times 10^6$. Earlier estimates based on

smaller samples of *O. rubiplumus* [$N_{ef} = 0.5 \times 10^6$, $n = (6)$] and *Osedax frankpressi* [$N_{ef} = 0.9 \times 10^6$, $n = (19)$] were lesser, but the present estimate is comparable to an estimate for *Osedax roseus* [$N_{ef} = 1.7 \times 10^6$, $n = (52)$] from Monterey Bay (Rouse *et al.* 2008). Based on published mtCOI sequences, we estimated $N_{ef} = 0.9 \times 10^6$ ($n = 18$) for *Osedax mucofloris* sampled off Sweden (Glover *et al.* 2005), but we were unable to estimate N_{ef} for *Osedax japonicus* as only one mtCOI sequence is publicly available (Fujikura *et al.* 2006). Nonetheless, all the available estimates are similar in magnitude, leading us to believe that local *Osedax* populations may generally be recruited from vast pools of larvae that represent the contributions of great numbers of females. We cannot tell from these unique geographical samples, however, the effective neighbourhood size of *Osedax* larval pools, the extent of geographical structuring, or the potential for isolation by distance. Indirect estimates of effective neighbourhood areas may eventually be possible with better sampling, but that will require the discovery or deployment of many more whale carcasses, difficult and expensive enterprises in oceanographic research (Smith & Baco 2003; Braby *et al.* 2007). Further inferences about the dispersal potential of *Osedax* may be advanced, however, from detailed knowledge of their larval biology and patterns of ocean currents.

The Monterey *O. rubiplumus* population does not appear to be at demographic equilibrium. Tajima's (1996) D was negative for the COI sequences (-2.389 ; $P < 0.01$). Although this statistic was developed to test selective neutrality, it is sensitive to departures from equilibrium caused by population expansions (Aris-Brosou & Excoffier 1996; Ray *et al.* 2003). The distribution of nucleotide mismatches (Fig. 4) also was consistent with expectations for a recent population expansion (Harpending 1994). We estimated that somewhere between 10^5 and 10^6 generations have passed since the beginning of this putative expansion, but we do not know the average generation time for *O. rubiplumus*, and we can only guess about mutation rates for mitochondrial COI in this species. Consequently, attempts to estimate time in years since the beginning of this expansion would be premature. Furthermore, the present genetic markers mutate far too slowly to assess more recent demographic events, such as potential decreases in *Osedax* population size that might have accompanied commercial whaling. These potential demographic losses may be buffered, however, if *Osedax* are able to colonize, grow and reproduce on the bones of smaller marine mammals (Jones *et al.* 2008; Vrijenhoek *et al.* 2008).

If female size can be taken as a surrogate for their age, *O. rubiplumus* sex ratios shifted dramatically from female biased to very strongly male biased. Immature, nonspawning females typically hosted no males in their tubes, and the number of males in these harems increased exponentially with female size. Size variation among males in the larger

harems suggests that the males were continuously recruited during the lifespan of a mature female (Fig. 1d). The smallest males were packed with yolk droplets; intermediate size males had diminished yolk and various stages of spermiogenesis; the largest and most elongate males had no yolk and masses of spermatids and mature sperm. We also observed occasional 'ghosts' of males that apparently were empty of yolk and sperm, and presumably were dead. Variance in males' sizes covaried closely with female sizes, and even the largest (presumably oldest) females had small males that appeared to be new recruits. The longevity of individual males remains unknown. They have no apparent means to feed and do not possess the bacterial endosymbionts (Oceanospirillales) found in *Osedax* females (S. Goffredi, Occidental College, Los Angeles, CA, personal communication). The males do not appear to be parasitic, but females might be able to nourish them in some way that remains to be discovered. For now, the simplest hypothesis is that these males simply deplete their yolk reserves to manufacture sperm and then die, while new males are continuously recruited from the larval pool. Although the present results are consistent with expectations for ESD, we cannot exclude a role for genotypic sex determination (GSD). Strict GSD involving a primary 1:1 sex ratio would require massive shifts in mortality to explain the changes in sex ratio observed in this study. However, a mixed strategy involving primarily ESD and a little GSD, as may exist in *Bonellia viridis*, could provide a demographic advantage in disturbed environments where females do not tend to saturate the available settlement sites (Berec *et al.* 2005).

Ghiselin (1974) predicted that male dwarfing due to early sexual maturation is more likely to evolve when resources are limited and when males do not compete directly for access to females. Male dwarfism reduces or nearly eliminates food competition with females that can attain greater body sizes and fecundities (Ghiselin 1974; Slatkin 1984; Shine 1989). The extreme subdivision of ecological roles seen in the *O. rubiplumus* sexes is clearly consistent with these ideas. Fertility selection probably favoured rapidly growing females that could efficiently exploit scattered and potentially ephemeral mammalian bones (Jones *et al.* 2008; Vrijenhoek *et al.* 2008). Simultaneously, natural selection probably favoured small, nonfeeding, and mobile males that breed at a very early age; achieved by transforming *Osedax* larvae into males. As we have seen *O. rubiplumus* males vary considerably in size (0.2–1 mm), but we have no evidence that male–male competition for access to eggs or sites of sperm deposition might act antagonistically towards selection for extreme dwarfism, as seen in an acanthocean barnacle and an orb-weaving spider (Gotelli & Spivey 1992; Foellmer & Fairbairn 2005). Such counter-pressures might help to maintain the larger size of *Bonellia viridis* males (1–3 mm), which are reported to be parasitic and live in small groups of three to four males inside the androecium, a modified

anterior portion of the nephridium (Jaccarini *et al.* 1983). It should be noted, however, that the dwarf males of *Osedax roseus* do not show comparably large size ranges, and they have much smaller harem sizes (Rouse *et al.* 2008). Perhaps the highly numerous males of *O. rubiplumus* compete to access sites for sperm transfer to the female, but the transfer mechanisms and the site of fertilization in *Osedax* have yet to be elucidated.

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