NEWS AND VIEWS

PERSPECTIVE

Between a whale bone and the deep blue sea: the provenance of dwarf males in whale bone-eating tubeworms

NOAH K. WHITEMAN

Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Abstract

When researchers first caught a glimpse of the lush carpet of pink tubeworms covering the scattered bones of a dead grey whale 2900 m below the surface of Monterey Bay, the excitement onboard the Western Flyer (the mother ship of the remotely operated vehicle the Tiburon) must have been electrifying. The discovery of a new genus and several species of whale bone-eating *Osedax* tubeworms (Annelida, Siboglinidae) a mere 6 years ago from the deep sea was itself noteworthy. But what the researchers peering into the video monitors aboard the Western Flyer could not have known at that moment was that in the gelatinous tubes of those worms clung even more peculiar forms: harems of tiny, paedomorphic males of *Osedax*, numbering in the hundreds at times. Whereas female tubeworms bore into the marrow of whale bones (possibly via enzymes from their endosymbiotic bacteria), the dwarf males secondarily colonize the tubes of the resident females. The number of males in a female's tube increases over time in a curvilinear fashion. Dwarf males are known from all *Osedax* species examined to date, yet the origin of the males was an open question. In this issue, Vrijenhoek et al. provide compelling evidence that dwarf males found in the tubes of female *Osedax* worms are derived from a common larval pool and are unlikely to be the sons of host females or the progeny of females in the local genetic neighbourhood. This study provides an important foundation for future work on the ecology and evolution of extreme male dwarfism in *Osedax* and sexual size dimorphism more generally.

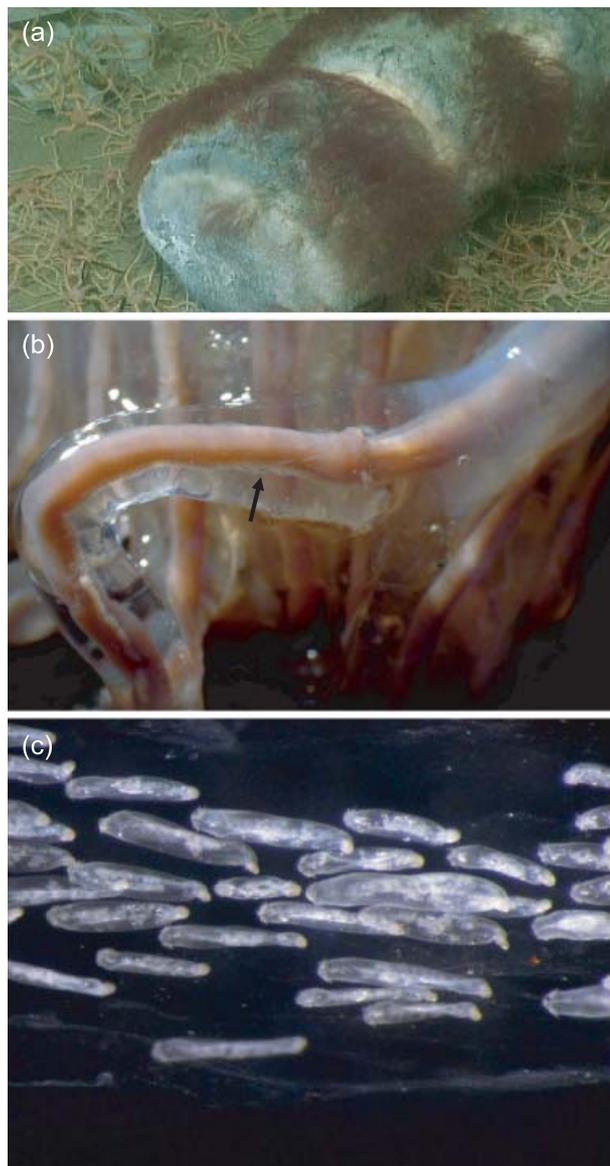Correspondence: Noah K. Whiteman, Fax: 617-495-5667; E-mail: nwhiteman@oeb.harvard.edu

Fig. 1 (a) Vertebrae of a grey whale carcass that was sunk by Vrijenhoek and colleagues at 1820 m depth in Monterey Bay. The bones are covered with female *Osedax* spp. and numerous brittlestars are on the ocean floor. Photo courtesy of Dr Robert Vrijenhoek and Monterey Bay Aquarium Research Institute. (b) Gelatinous tube of a female *Osedax* sp. individual with male harem inside tube (harem is indicated by a black arrow). Photo courtesy of Dr Greg Rouse. (c) Close-up view of a male harem inside a gelatinous tube of a female *Osedax* sp. individual. Photo courtesy of Dr Greg Rouse.

'Blest be the man that spares these stones,
And curst be he that moves my bones.'
— Partial inscription on the tomb of William Shakespeare, Stratford-Upon-Avon, UK —

The evolution of extreme dwarfing in males, where males are 50% smaller than females, has puzzled biologists ever since Darwin discovered dwarf males in barnacles (Crustacea, Cirripedia) (Darwin 1851). Since then, a number of cases of extreme male dwarfism have been found in a variety of taxa across a wide swath of the tree of life, including from arachnids, lophiiform anglerfishes, cephalopods, crustaceans, cycliophorans, rotifers and gastropods (Vollrath 1998). Ghiselin (1974) suggested that dwarf males were often found in species where females were sedentary or hard to find and natural selection was likely to have been the predominant force driving male dwarfism (Vollrath 1998). Indeed, female *Argonauta* octopuses, which live in the open ocean, or pelagic female *Linophryne* anglerfishes, which are found below 1000 m depth, must be challenging for a mate to find. However, it is difficult for one to imagine a more ephemeral or patchy resource than the gelatinous tube of a female *Osedax* tubeworm that lives in a whale bone on the ocean floor (Fig. 1). Yet, this is where mature (paedomorphic) male *Osedax* tubeworms reside.

Described only 6 years ago (Rouse *et al.* 2004), *Osedax* tube worms comprise *c.* 10 known and undescribed species (Vrijenhoek *et al.* 2008b) of annelid tubeworm (Siboglinidae). Females are known only from submerged mammalian bones and environs and bore into bones with a ramifying root. Endosymbiotic bacteria (Gammaproteobacteria: Oceanospirillales) living in bacteriocytes in the ramifying roots of mouthless and gutless female tubeworms may help digest bone tissue and provide energy for the host (Goffredi *et al.* 2005, 2007). Successional waves of sympatric *Osedax* species displace one another on whale bones in Monterey Bay (bones may persist for only a few years), suggesting that females of each species have a relatively short window of time in which to reproduce. Dwarf males have been found in all *Osedax* species that have been examined, but in no other siboglinid worms, and despite morphological differences between species, males all appear to retain characters of siboglinid larvae at the trochophore stage (Rouse *et al.* 2004, 2008). Males feed off yolk droplets and are typically replete with spermatozoa.

Rouse *et al.* (2004) hypothesized that environmental sex determination (ESD) is operating in *Osedax* species. The conceptually simple scenario is as follows. When a larva settles on a whale bone, it turns into a female; when a larva settles on or in a female tubeworm, it turns into a male. The working hypothesis is that the ephemeral nature of whale bones as a food resource, coupled with the unpredictable locations of the whale falls and the competition and succession among *Osedax* species has driven the extreme sexual size dimorphism and the evolution of ESD in these taxa.

To date, there is no direct evidence for ESD in *Osedax* worms. As a first step towards testing this hypothesis, Vrijenhoek and colleagues, in this issue of *Molecular Ecology*, examined male recruitment in *Osedax rubiplumus* in Monterey Bay and proposed three reasonable alternative scenarios that could explain the provenance of the male clusters (harems) in the tubes of females:

(i) *arrhenotoky*, in which males are derived from the female host (parthenogenesis), (ii) *common larval pool*, in which males are derived from the same larval pool as females, (iii) *neighbourhood*, in which males are derived from the local genetic neighbourhood (e.g. worms on that whale carcass or a nearby carcass).

To test these hypotheses, Vrijenhoek and colleagues first developed a set of predictions that would allow objective evaluation of the three scenarios presented above. They then collected *Osedax* females from two whale carcasses on the floor of Monterey Bay using the ROV Tiburon, including one found *in situ* and one that was towed out into the bay and sunk. Females were dissected, allowing precise assignment of each set of males with a particular female. Body size of males and females was measured, and a series of males and their female 'hosts' was genotyped at a mitochondrial locus (cytochrome *c* oxidase subunit I.) from each whale carcass and at a single allozyme locus from one of the carcasses.

Interestingly, most males in a harem were found next to the female's oviduct, attached with their chaetal hooks to the female's tube (and not to the female's body). Harem size was positively correlated with female trunk width; the largest harem comprised 607 males from a single female. Based on indirect evidence (the degree of variation in male body size), Vrijenhoek and colleagues suggest that males appear to colonize the tubes of females throughout the females' life and this is consistent with circumstantial evidence from studies involving a sympatric congener (Rouse *et al.* 2008), suggesting that acquiring males is a general phenomenon among female *Osedax* species.

The genetic evidence showed two main patterns. First, based on a single allozyme locus, there was no evidence of nonrandom mating among tubeworms. This is the most tenuous of the conclusions because more loci are needed before characterization of the mating system is justified. In particular, it would be extremely interesting to determine whether reproductive skew decreases with the age of the female (as the number of males in a tube increases). The mitochondrial data suggest a very large inbreeding effective population size for female *O. rubiplumus*, with little evidence of genetic drift playing a dominant role in the recent history of this genome. Although there is no calibrated synonymous mutation rate for *Osedax* mitochondria, Vrijenhoek and colleagues used a range of rates to estimate the effective size of the source of the larval pool, which may be on the order of one million individuals. Rough estimates of population expansion indicate that the population had recently been through a demographic expansion.

Mitochondrial diversity indices were essentially identical for male and female worms. Haplotype networks showed no clustering of haplotypes by sex, nor was there any significant mitochondrial structure present between male and female samples in the neighbourhood or between whale carcasses. Considering harems where at least seven males were genotyped, males in 50% of these harems did not share the host female's mitochondrial genotype, and the remaining 50% where at least one male did share the host female's haplotype were likely due to chance (the authors used the binomial distribution to test this). Similarly, no evidence for co-ancestry was found between harems in the same neighbourhood. Thus, the only hypothesis that could not be rejected, based

on this evidence, was the *common larval pool*. Here again, though, finer-scale markers, such as microsatellites, would provide more informative measurements of population structure, across spatial scales.

Is it possible to infer changes in whale population size via changes in *Osedax* population sizes? In other words, might whale-fall associates be used as proxies (Whiteman & Parker 2005) for recent whale population histories (see Kaliszewska *et al.* 2005 for a case study involving cyamid whale 'lice')? Such inferences are likely to be extremely challenging. First, mutation rates at mitochondrial loci are too slow to reflect the demographic signal that recent whaling might have had on effective population sizes of *Osedax* worms (see Alter *et al.* 2007). More importantly, however, experimental and observational data suggest that *Osedax* worms are capable of colonizing and living on submerged bones of mammal species other than whales, including those of cows (Jones *et al.* 2008; Vrijenhoek *et al.* 2008a), although the subject is controversial (Glover *et al.* 2008). The addition of nuclear data, coalescent simulations and finer-scale markers could test the hypothesis that *Osedax* worms, and other species that depend on large cetaceans (Dahlgren *et al.* 2004), experienced population size flux in the last 200 years. Nonetheless, if *Osedax* species are not whale-fall specialists, their population size fluctuations are unlikely to be tightly correlated to those of the great whales. A survey for *Osedax* spp. on naturally occurring vertebrate falls (other than those of great whales) on the seafloor would help address the resource breadth controversy.

There are several other key questions about *Osedax* natural history that remain unanswered. Ongoing genetic studies of *Osedax* spp. (Vrijenhoek, personal communication) are aimed at investigating gene flow among whale carcasses and assessing mating systems (e.g. simultaneous polyandry). Other studies, including determining the site and mode of fertilization, testing the ESD hypothesis, identifying the mechanisms underlying larval location of mammalian bones, and testing whether sympatric forms that successively colonize a whale carcass represent adaptive radiations, are also underway (Vrijenhoek, personal communication).

The fresh insight provided into our understanding of male recruitment in these most unusual worms illustrates how combining detailed life history and ecological data with genetic tools can provide unprecedented insight into the marvelous lives of even the most cryptic and far-flung species on our planet.

Acknowledgements

References

Alter SE, Rynes E, Palumbi SR (2007) DNA evidence for historic population size and past ecosystem impacts of gray whales. *Proceedings of the National Academy of Sciences, USA*, **104**, 15162–15167.

Dahlgren TG, Glover AG, Baco A, Smith CR (2004) Fauna of whale falls: systematics and ecology of a new Polychaete (Annelida: Chrysopetalidae) from the deep Pacific Ocean. *Deep Sea Research. Part 1. Oceanographic Research Papers*, **51**, 1873–1887.

Darwin C (1851) *A Monograph on the Cirripedia*. Royal Society, London.

Ghiselin M (1974) *The Economy of Nature and the Evolution of Sex*. University of California Press, Berkeley, California.

Glover AG, Kemp KM, Smith CR, Dahlgren TG (2008) On the role of bone-eating worms in the degradation of marine vertebrate remains. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1959–1961.

Goffredi SK, Orphan VJ, Rouse GW *et al.* (2005) Evolutionary innovation: a bone-eating marine symbiosis. *Environmental Microbiology*, **7**, 1369–1378.

Goffredi SK, Johnson SB, Vrijenhoek RC (2007) Genetic diversity and potential function of microbial symbionts associated with newly discovered species of *Osedax* worms. *Applied and Environmental Microbiology*, **73**, 2314–2323.

Jones WJ, Johnson SB, Rouse GW, Vrijenhoek RC (2008) Marine worms (genus *Osedax*) colonize cow bones. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 387–391.

Kaliszewska ZA, Seger J, Rowntree VJ *et al.* (2005) Population histories of right whales (Cetacea: *Eubalaena*) inferred from mitochondrial sequence diversities and divergences of their whale lice (Amphipoda: *Cyamus*). *Molecular Ecology*, **4**, 3439–3456.

Rouse GW, Goffredi SK, Vrijenhoek RC (2004) *Osedax*: bone-eating marine worms with dwarf males. *Nature*, **305**, 668–671.

Rouse GW, Worsaae K, Johnson SB *et al.* (2008) Acquisition of dwarf male 'harems' by recently settled females of *Osedax roseus* n. sp. (Siboglinidae; Annelida). *Biology Bulletin*, **214**, 67–82.

Vollrath F (1998) Dwarf males. *Trends in Ecology & Evolution*, **13**, 159–163.

Vrijenhoek RC, Collins PC, Van Dover CL (2008a) Bone-eating marine worms: habitat specialists or generalists? *Proceedings of the Royal Society B: Biological Sciences*. Published Online, doi: 10.1098/rspb.2008.0350.

Vrijenhoek RC, Johnson SB, Rouse GW (2008b) Bone-eating *Osedax* females and their 'harems' of dwarf males are recruited from a common larval pool. *Molecular Ecology*, **17**, 4535–4544.

Whiteman NK, Parker PG (2005) Using parasites to infer host population history: a new rationale for parasite conservation. *Animal Conservation*, **8**, 175–181.