Coral growing on North Sea oil rigs

These installations are home to thriving colonies of an endangered cold-water coral.

This summer the coral *Lophelia pertusa* was found growing on oil platforms in the North Sea and on the Brent Spar oil-storage buoy during its decommissioning. The findings indicate that *Lophelia* has a wider distribution and a more rapid rate of growth than previously believed. The discovery also has implications for the debate over oil exploration in the Atlantic Ocean and the perceived benefits of onshore dismantling of deep-water platforms.

When Brent Spar was raised out of the sea in stages for dismantling (Fig. 1), colonies of *Lophelia* of up to 20 cm linear growth were found on the sides and bottom, which had been at depths of 60–109 m. *Lophelia* has recently been filmed at depths of 100–129 m on two platforms in the North Sea about 140 km east of the Shetland Islands. These domed colonies were up to 54 cm long, and were found on installations that have been producing oil since the late 1970s. Annual average linear growth rates of 25 mm have previously been suggested for this coral[1], but more recent estimates of 5.5–6.0 mm have been proposed[2]. The size of the colonies on these 20-year-old platforms means that the average growth rate must have been at least 26 mm per year. If the colonies found on Brent Spar had grown at a similar rate, then *Lophelia* must have settled on the structure when it was in the Brent Field in the North Sea.

Lophelia is widespread in the Atlantic at depths of 150–1,500 m, but is particularly common on the upper continental slope at 200–600 m (refs 3,4). It has also been found at a depth of 50 m in Scandanavian fjords[5], off the coast of Norway[6], and on the Beryl platform[7]. Its occurrence on oil installations is the first recorded instance of live colonies of this species in the North Sea. It has been shown[2] that *Lophelia* occurs at temperatures of 4–8 °C. At the original Brent Spar site (61° 03′ N, 01° 40′ E), the water temperature at the depth where the shallowest *Lophelia* were found can exceed 10 °C (ref. 8); in the area of the two production platforms, the maximum water temperature at depths of 100–129 m varies between 7.6 and 9.7 °C.

The presence of *Lophelia* on oil-producing platforms has implications for the licensing of oil exploration. Greenpeace contends that the British Department of Trade and Industry failed to consider whether licensed oil-producing areas that may contain *Lophelia* should be designated as potential special areas of conservation and, as such, is in contravention of the European Commission's Species Habitat Directive (92/43/EEC).

Figure 1 The top of Brent Spar being removed from the Heerema heavy-lift barge during its dismantling in Yrkefjorden, Norway.

It has been suggested that *Lophelia* is susceptible to disturbance from increased sedimentation and from the toxicological effects of drilling discharges[9,10]. However, the apparently healthy colonies of *Lophelia* on platforms in the North Sea have been exposed to agreed quality standards of operational discharges, such as oily water, drilling muds and chemicals, and contaminants that may leak from the cuttings piles (E. Breuer, personal communication) that lie 10–15 m below some of the colonies. This indicates that it is not obviously affected by discharges from oil platforms.

The occurrence of the coral raises questions about how to deal with this species, which is listed under the Convention on International Trade in Endangered Species (CITES), when platforms are decommissioned. At a meeting in Sintra in 1998 of countries belonging to the Oslo–Paris (Ospar) convention on protecting the marine environment, Ospar decision 98/3 indicated that the 'footings' of large platforms (jacket weight of more than 10,000 tonnes) might be left in place. Such an option would preserve existing colonies and might allow *Lophelia* to spread in the North Sea.

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Evolution

Conservation of a sex-determining gene

Vertebrates exhibit a surprising array of sex-determining mechanisms, including X- and Y-chromosome heterogametes in male mammals, Z- and W-chromosome heterogametes in female birds, and a temperature-dependent mechanism in many reptiles[1]. The Y-chromosome-linked *SRY* gene initiates male development in mammals[2,3], but other vertebrates lack *SRY* and the genes controlling sex determination are largely unknown. Here we show that a gene implicated in human testis differentiation, *DMRT1*, has a gonad-specific and sexually dimorphic expression profile during embryogenesis in mammals, birds and a reptile (*Alligator mississippiensis*). Given the different sex-determining switches in these three groups, this gene must represent an ancient, conserved component of the vertebrate sex-determining pathway.

The human *DMRT1* gene was isolated by virtue of its homology to the *doublesex* gene from *Drosophila* and *mab-3* from *Caenorhabditis elegans*, which encode putative transcription factors with a conserved DM domain that is thought to bind DNA[4]. *DMRT1* maps to the minimal region of the small arm of human chromosome 9, which is deleted in patients with XY male-to-female sex reversal[4–6]. A related gene, *DMRT2*, has also been mapped within this region[7]. Human genetic data indicate that *DMRT1*, alone or together with *DMRT2*, may operate in a dose-dependent fashion within the male (testis)-determining pathway. The chicken *DMRT1* homologue is located on the Z sex chromosome[8].

We used regions mainly or totally outside the DM domain of the published human and chicken *DMRT1* complementary DNA sequences to design primers for amplification, by the polymerase chain reaction with reverse transcription (RT-PCR), of the mouse, chicken and alligator homologues. Sequence analysis confirmed the identity of the PCR products (GenBank accession numbers for mouse and alligator

DMRT1 are AF192561 and AF192560, respectively). Specific mouse and chicken PCR fragments were used as probes for whole-mount *in situ* hybridization analysis of *DMRT1* expression during urogenital development. These fragments detected single bands on Southern blots of genomic DNA, excluding the possibility of cross-hybridization with *DMRT2*. For the alligator, specific primers were used for RT-PCR analysis of whole urogenital tissues or gonads from embryos incubated at 30 °C (female-producing), 33 °C (male-producing) and 34.5 °C (female-producing).

Expression of *DMRT1* was sexually dimorphic in all three species, and was stronger in male gonads than in female ones (Fig. 1). In mouse embryos at 11.5 days post-coitum (d.p.c.), when the testis-determining *Sry* gene is activated in males and just before testicular differentiation, *DMRT1* was already being expressed in the gonads of both sexes. By 14.5 d.p.c., *DMRT1* was expressed more strongly in differentiating testes than in ovaries (Fig. 1a). Taken together with human gene results implicating *DMRT1* in XY sex reversal, these observations indicate that *DMRT1* is necessary for testis differentiation, presumably lying downstream of the master male-determinant *SRY*.

In chickens and other birds, the mechanism of sex determination is unknown. Sex may be controlled by a dominant ovary-determining gene carried on the W chromosome, or it may depend on Z-chromosome dosage (two doses for male development and one for female). *DMRT1* is Z-linked, and its expression profile implicates it in avian sex determination. As in the mouse, chicken embryos showed gonad-specific expression of *DMRT1*, with much stronger expression in developing male than in female gonads (Fig. 1b). This difference was evident before and during the time of gonadal sex differentiation (developmental stages 25–30; days 4.5–6.5 of incubation).

In birds, the Z chromosome does not seem to undergo inactivation, as the X chromosome does in mammals. Although the sexually dimorphic expression of *DMRT1* in chicken embryos may simply reflect this lack of dosage compensation, expression in the male is clearly more than twice that seen in females and suggests strong upregulation in ZZ embryos. This observation, together with the fact that expression is confined to the gonads, points to an active role for *DMRT1* in avian gonadal development. In the alligator, *DMRT1* expression was initially detectable by RT-PCR in the urogenital systems of embryos incubated at both male- and female-producing temperatures (Fig. 1c). However, gonadal expression subsequently became higher in developing male embryos than in female embryos (Fig. 1c).

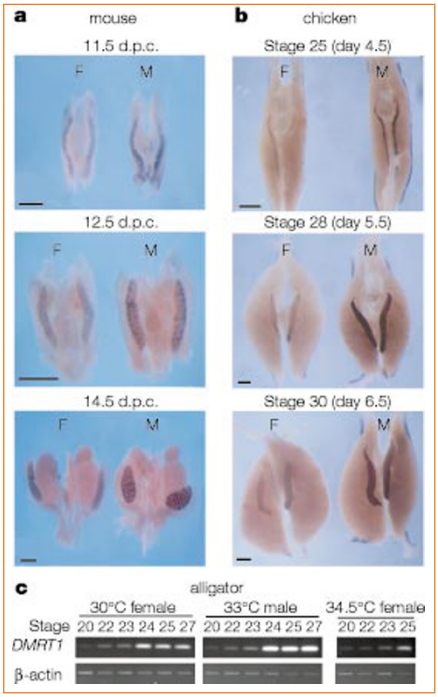

Figure 1 Sexually dimorphic expression of *DMRT1* in embryonic gonads. **a, b,** Whole-mount *in situ* hybridization of embryonic mouse (**a**) and chicken (**b**) urogenital systems during development, showing stronger expression (purple staining) in male than in female gonads (scale bar, 0.5 mm). **c,** RT-PCR analysis of embryonic alligator urogenital systems (developmental stages 20–23) or isolated gonads (stages 24–27) showed more upregulation of *DMRT1* expression at the male-determining temperature (33 °C) than at either female-producing temperature. β-Actin gel-loading controls are also shown.

The sexually dimorphic pattern of *DMRT1* expression in mouse, chicken and alligator embryos is consistent with a conserved role in vertebrate sex determination. We suggest that high *DMRT1* expression is necessary for testicular differentiation, whereas lower expression is compatible with ovarian differentiation. Our observations support the idea that core components of the vertebrate sex-determining pathway have been conserved during evolution. As *Drosophila* and *C. elegans* also have related genes involved in sexual development[4], the DM-domain genes are the first to show sexually dimorphic expression across both vertebrate and invertebrate phyla.

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Bioenergetics

Proton pumping by cytochrome *c* oxidase

Proton pumping by cytochrome *c* oxidase[1] was thought to be restricted to the oxidative part of its catalytic cycle[2], but this has been questioned[3]. New results[4] were interpreted as an indication that two protons are pumped during the oxidative phase, and two during a subsequent reductive phase, and that this latter pumping is energetically coupled to the oxidative phase. Here I re-evaluate these results and draw an alternative conclusion.

Figure 3 of ref. 4 shows that only about 44% of the electric-field generation (caused by proton pumping, electron transfer and proton uptake) occurs during oxidation of the fully reduced enzyme. However, the claim[4] that four protons (two during oxidation and two during re-reduction) are pumped per catalytic cycle is inconsistent with the other experiment reported at the same time (Fig. 2 of ref. 4).

There are four indications that a molar excess of oxygen over active, correctly orientated cytochrome *c* oxidase has been used (www.biophys.mpg.de/michel/public/cox-disc). For example, complete oxidation leads to pumping of only about 1.2 protons per enzyme. Re-reduction would then lead to pumping of 2.4 protons per turnover, assuming that the same number of protons were pumped during re-reduction as during oxidation. Four protons per enzyme can then be pumped, if a molar excess of oxygen is present, leading partially to a second turnover.

Assuming an optical pathway of roughly 1 cm (ref. 5) and using published spectra[6], we calculate that the concentration of oxidized cytochrome *c* oxidase (Fig. 2a, bottom, of ref. 4) is roughly 0.70 μM, rather than 1.25 μM. The addition of 1.25 μM O_2 was superstoichiometric. Another possibility is that the optical path length might have been shorter (0.56 cm), but calculations for Fig. 2 of ref. 5 indicate a longer path length (0.73 cm) for the device used.

The observation that four protons have already been pumped when only 40% of the haem groups have been re-reduced was interpreted[4] as an indication that input of only one electron may be sufficient to elicit pumping of two protons, and was presented as support for the existence of an energy-rich state, O~. However, this result was caused by the excess of oxygen, so there is no evidence that four protons are pumped per turnover when starting from the fully reduced enzyme, or for the existence of the O~ state. The fully reduced state is not part of the natural reaction cycle of cytochrome *c* oxidase. This artificial cycle tells us little about proton pumping in the natural cycle,