

The search for *Wolbachia*

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Introduction.

Wolbachia is an intracellular symbiont of invertebrates, with a very broad host range. It belongs to the Rickettsia-like bacteria, grouping within the α -Proteobacteria (O'Neill et al. 1992; Zhou et al. 1998). Infections with *Wolbachia* lead to various reproductive abnormalities in the host. Examples are parthenogenesis in wasps or feminization of genetic males in one isopod species. Since many marine invertebrates are known to have endosymbionts, we wanted screen for the presence of *Wolbachia* in several marine invertebrates. The availability of a specific 16S rRNA *Wolbachia* primer and a primer based on a *Wolbachia* surface protein gene enabled us to screen marine invertebrates using PCR amplification of genomic DNA.

Materials and methods.

DNA was extracted from the following samples:

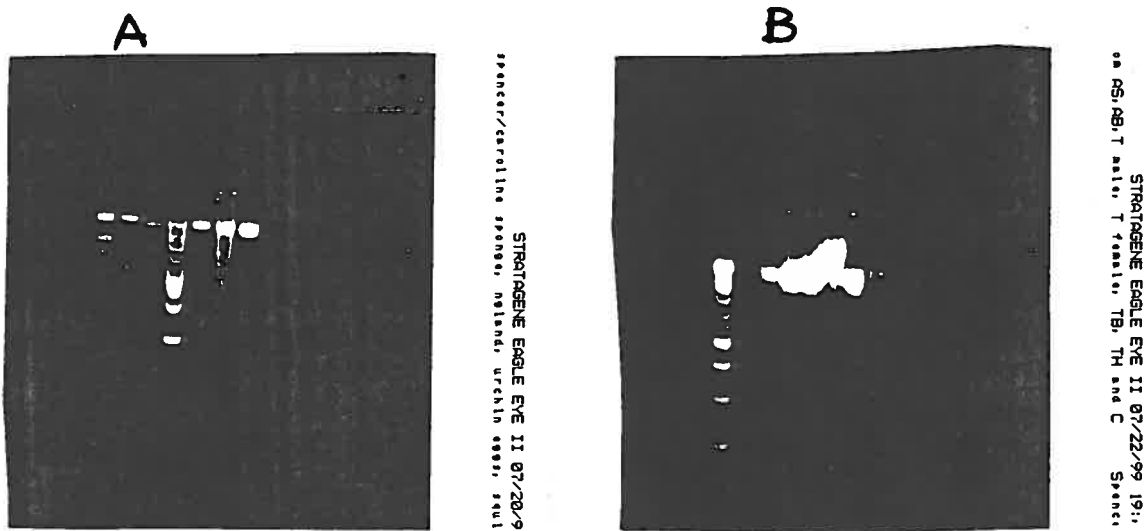
Eggs from Sea Urchin *Lytichina* spp. (UE)
Eggs from Squid *Loligo peali* (SE)
Loligo peali nidamental gland (NG)
Loligo peali accessory nidamental gland (ANG)
Salt Pond Sediment (SPS)
Sponge *Microciona* (Sponge)
Big Ant (AB)
Small Ant (AS)
Mussel *Modiolus demissus* gut (MG)
Polychaeta worm *Chaetopterus variopedatus* sperm (TM)
Chaetopterus variopedatus eggs (TF)
Termite *Reticulotermes flavipes* body (TB)
Reticulotermes flavipes hindgut (TH)
Crab eggs (C)

DNA was extracted using the MoBio UltraClean Soil DNA Isolation Kit.

PCR amplifications were done in 20 μ l reaction volumes containing:
 13.5 μ l dH₂O, 2 μ l buffer (10 x), 2 μ l mMgCl₂ (25 mM), 0.5 μ l dNTP's, 0.5 μ l forward primer (20 mM),
 0.5 μ l reverse primer (20 mM) and 1 unit of Taq polymerase.
 The thermal profile used was: 1 min 94°C, 55°C 1 min, 72°C 1 min for 35 cycles
 The primers used were described previously by O'Neill et al. (1992) and Zhou et al. (1998).

The primers were kindly provided by Scott O'Neill (New Haven, CT).

Results and discussion.



Figures 1: Results of the DNA extraction .

Lanes contain DNA from : UE; SPS; Sponge; "ladder"; AB; SE; NG (A)
 "ladder"; AS; AB; TM; TF; TB; TH; C (B)

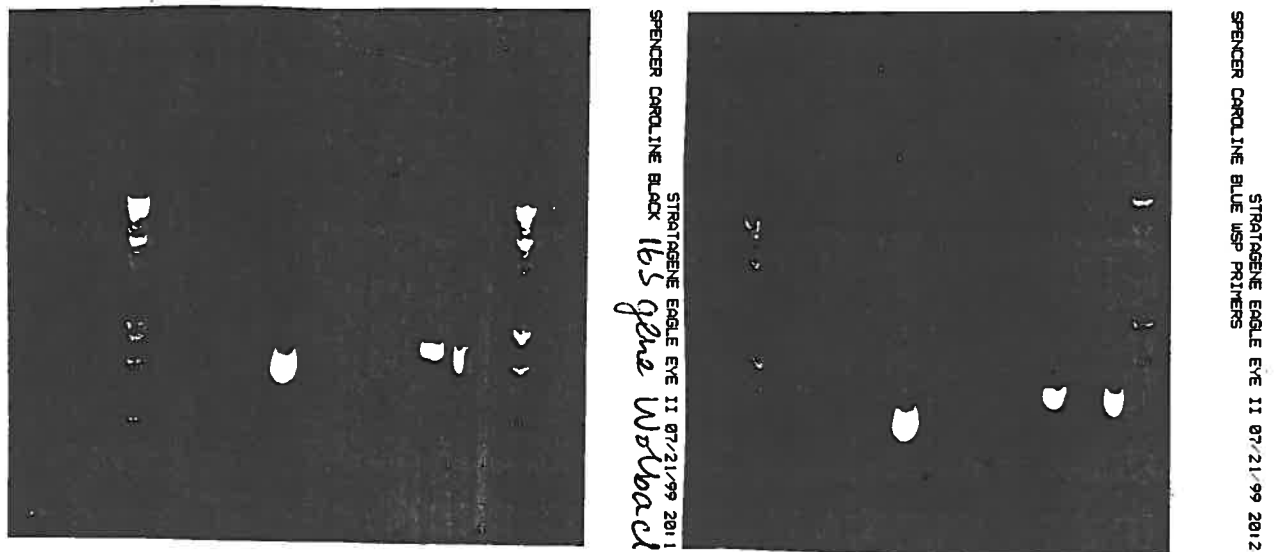


Figure 2: PCR assay with specific 16S rRNA primer (A) and *wsp* primer (B).

Lanes contain PCR product from: "ladder"; UE; SE; SPS; Sponge; AB; NG; ANG; SG; MG;
 positive control; negative control; "ladder"

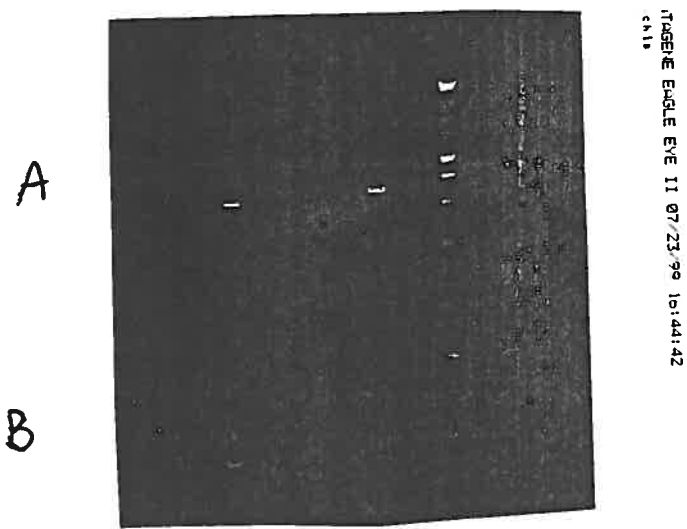


Figure 3: PCR assay with specific 16S rRNA primer (A) and *wsp* primer (B).
 Lanes contain: AB; AS; AB; TM; TF; TB; TH; C; positive control; negative control; "ladder".

Both figure 2 and 3 show only a PCR product from the big ant, indicating the presence of *Wolbachia*. A recent paper by Wenseleers et al (1998) reports on the presence of *Wolbachia* in ants. Out of 50 Indo-Australian species, 50% screened positive for *Wolbachia*, which is an unusual high incidence. Our results confirm that *Wolbachia* is present in ants. However, no *Wolbachia* could be found in any of the marine invertebrates with the methods we used.

Literature.

1. O'Neill, S.L., Giordano, R., Colbert, A.M.E., Karr, T.L. & Robertson, H.M. (1992) 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl. Acad. Sci.* 89: 2699-2702.
2. Wenseleers, T., Ito, F., Van Borm, S., Huybrechts, R., Volckaert, F., & Billen, J. (1998). Widespread occurrence of the microorganism *Wolbachia* in ants. *Proceedings of the Royal Society of London - Series B: Biological Sciences*, 265: 1447-52.
3. Zhou, W., Rousset, F., & O'Neill, S. (1998) Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proceedings of the Royal Society of London - Series B*: 265: 509-515.