# The origin and regeneration of free-living Fucus vesiculosus in the Baltic <sup>1,2\*</sup>Roxana Preston, <sup>3</sup>Perttu Seppä, <sup>4,5</sup>Ellen Schagerström, <sup>1</sup>Jaanika Blomster

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## INTRODUCTION

*Fucus vesiculosus* is one of only a few canopy forming, perennial macroalgae providing the foundations for highly productive ecosystems within the Baltic Sea. Two forms have been documented, as either attached or free-living, yet the connection between the two are poorly understood. It has long been assumed that free-living populations originate from the attached form, whereby removed pieces of thalli from attached populations collect in still locations and persist over time. Using genetic techniques we investigate the validity of this assumption and delve into how the free-living populations are maintained once formed.

## METHODS

Mitochondrial barcode sequencing<sup>(1)</sup> Microsatellite genotyping<sup>(2)</sup>

Six subbasins: 14 sympatric sites 7 allopatric sites

100 thalli sequenced 1443 thalli genotyped

140 A T A C C G G A AG T G A G A A T A C T T G C A T G A G

mmh

(<sup>1)</sup> Coyer, J.A., Hoarau, G., Oudot-Le Secq, M.P., Stam, W.T. and Olsen, J.L., 2006. A mtDNA-based phylogeny of the brown algal genus Fucus (Heterokontophyta; Phaeophyta). Molecular phylogenetics and evolution, 39(1), pp.209-222. <sup>(2)</sup> Engel, C.R., Brawley, S.H., Edwards, K.J. & Serrão, E. 2003. Isolation and crossspecies amplification of microsatellite loci from the fucoid seaweeds Fucus vesiculosus, F. serratus and Ascophyllum nodosum (Heterokontophyta, Fucaceae). Mol. Ecol. Notes. 3:180–2.; Perrin, C., Daguin, C., Vliet, M. Van De, Engel, C.R., Pearson, G.A. & Serrão, E.A. 2007. Implications of mating system for genetic

diversity of sister algal species: Fucus spiralis and Fucus vesiculosus (Heterokontophyta, Phaeophyceae). Eur. J. Phycol. 42:219–30.

## **ORIGIN & REGENERATION**



network. Circle size is relative to number o ppies present. A branch represents a genetic distance and hash marks represent a single mutation. Dashed lines represent genetic distance between unlinked haplotypes. [sequencing]

Within Askö, Seili and Tvärminne the forms generally group more closely together (Figure 2) suggesting some level of geneflow. The other subbasins did not form consistent clusters. The overall close grouping indicates that the two forms are the same species.

Six mitochondrial haplotypes were found in the Baltic population, with the forms sharing the two most dominant haplotypes (H-I, H-VI). The dominant haplotype (H-I) is found in all subbasins and is suggested as the putative ancestral haplotype for the Baltic population (Figure 1).



Cumulative % of variance: 33.254 (axis 1: 17.522; axis 2: 15.731)

Connectivity is partially limited between forms (Table 1). Population structuring occurs among forms, but to a greater extent among populations within forms. When analysed separately, the freeliving form was highly structured (Rho<sub>sc</sub> 0.214<sup>\*\*\*</sup>) indicating that once formed free-living populations are more isolated.

| Table 1: Hierarchical AMOVA comparing the populati  | ion structure |
|---|---------------|
| of both forms. Populations are nested within forms. | [genotyping]  |

| of both forms. Populations are nested within forms. [genotyping] |                          |  |      |          |       |       |  |
|--|--------------------------|--|------|----------|-------|-------|--|
|  |                          | Variance                                     | df   | % of     | F-    | р     |  |
|  |                          | explained by                                 |      | variance | index | value |  |
|  | <b>Rho</b> <sub>ST</sub> | Within<br>population                         | 1194 | 0.815    | 0.185 | -     |  |
|  | <b>Rho</b> sc            | Among<br>population<br>nested within<br>form | 32   | 0.173    | 0.175 | 0.001 |  |
|  | <b>Rho</b> <sub>CT</sub> | Among forms                                  | 1    | 0.013    | 0.013 | 0.001 |  |
|  |                          |  |      |          |       |       |  |

Clonality is abundant in all free-living populations despite being nearly entirely absent in attached populations (Figure 3). The frequency of clonal multilocus genotypes (MLGs) in free-living populations varied across populations (range: 13–84%). Free-living populations are often maintained by high levels of asexual reproduction through clonal growth.



Figure 4: Proportions of shared clonal lineages in the study populations. Black represents unique multilocus genotypes and white represents clonal lineages unique to each site. Coloured portions represent clonal lineages shared between sampling sites. Numbers in white portions indicate the number of nonshared clonal genotypes represented. Site abbreviations: A, attached; F, free-living; AS, Askö; HS, Hiddensee; SA, Saaremaa; SE, Seili; TZ, Tvärminne. [genotyping]



Figure 3: Proportion of multilocus genotypes (MLGs) found within the free-living *Fucus vesiculosus* populations. Dark blue, clonal MLGs; light blue, unique MLGs. [genotyping]

Clonal MLGs are shared among populations from the same subbasin (Tvärminne, Saaremaa, Askö), but never across subbasins (Figure 4). Attached populations at sympatric sites do not supply material to their free-living conspecifics. This suggest that clonal growth of free-living thalli plays an important role in maintaining populations.

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