

Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*

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Abstract

Reproduction of attached large brown algae is known to occur only by sexual zygotes. Using microsatellites we show evolution of asexual reproduction in the bladder wrack promoting population persistence in the brackish water Baltic Sea (< 6 psu). Here a dwarf morph of *Fucus vesiculosus* is dominated by a single clone but clonal reproduction is also present in the common form of the species. We describe a possible mechanism for vegetative reproduction of attached algae, and conclude that clonality plays an important role in persistence and dispersal of these marginal populations, in which sexual reproduction is impaired by low salinity.

Keywords: adventitious branches, Baltic Sea, brackish water, brown macroalgae, clonal reproduction, *Fucus vesiculosus*, geographical parthenogenesis, marginal habitat

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Introduction

Numerous multicellular plants and algae, and some animal taxa, have the capacity for both sexual and asexual reproduction either within species or among closely related species. The secondary evolution of asexual reproduction within lineages in which sexual reproduction is considered ancestral is a challenging focus in evolutionary ecology, shedding light on the paradox of sex, for example (Kramer & Templeton 2001). A key pattern in many of these species is that asexual reproduction becomes more important towards geographical or ecological margins of species' distributions (Eckert 2001; Billingham *et al.* 2003; Kearney 2003). The Baltic Sea is a unique marginal marine ecosystem, as it is the largest brackish sea on Earth. It is moreover very recent in its present form, having remained at a low salinity since 3500 BP, after a major decrease in salinity resulting in the disappearance of many marine species that colonized the sea during the *Littorina* period (8000–4000 BP) (Ignatius *et al.* 1981; Russell 1985). Seagrasses and red algae that possess both sexual and asexual reproductive modes become increasingly asexual towards the inner parts of the

Baltic Sea (Reusch *et al.* 2000; Gabrielsen *et al.* 2002; Olsen *et al.* 2004), indicating a general problem for macro algae to reproduce sexually in the low-salinity environment of the Baltic.

Large brown seaweeds of the genus *Fucus* are key ecosystem-structuring species (Wallentinus 1991; Norton 1994; Chapman 1995). The life cycle of *Fucus* is diplontic (South & Whittick 1987) with sexual zygotes as the only known source of new attached algae (Serrão *et al.* 1999). The bladder wrack *Fucus vesiculosus* is the only large brown alga presently occurring in most of the Baltic Sea, to salinities of 3–4 practical salinity units (psu) in the Gulfs of Bothnia and Finland (Waern 1952; Kautsky *et al.* 1992; Serrão *et al.* 1996; Ruuskanen & Bäck 1999). At a salinity of 5 psu a dwarf morph occurs in sympatry with the common morph, but only the dwarf morph persists in areas of lower salinity. The dwarf morph is smaller and has a less wide thallus than the common morph (Bergström *et al.* in prep.). As fucoids have previously been known to reproduce only sexually, we did not expect to find asexual reproduction in Baltic populations of *F. vesiculosus*. However, we show here that the dwarf, low-salinity morph is dominated by clonal reproduction, and that clonality is also present in Baltic populations of the common morph. We conclude that asexual reproduction plays an important role in

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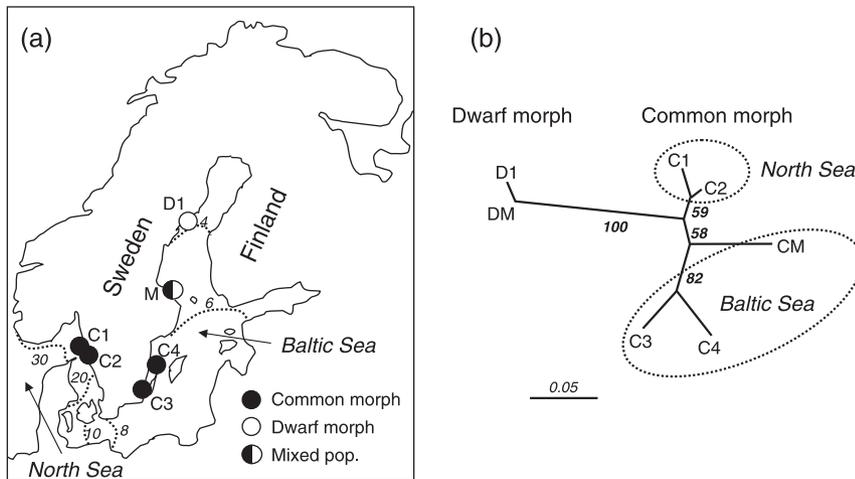


Fig. 1 Genetic relationships between populations of *Fucus vesiculosus*. DM and CM are samples of the dwarf and common morphs, respectively, from the mixed population (M). Dotted lines on the map (a) are isohalines with salinities shown above in psu. Values on the tree nodes (b) indicate the bootstrap percentage.

persistence and dispersal of Baltic Sea populations of *Fucus*, where sexual reproduction is impaired by low salinity.

Materials and methods

Genetic analysis

We used five microsatellite loci (L20, L38, L58, L85, L94 as has been previously described by Engel *et al.* 2003) to score genetic variation within and among populations of *Fucus vesiculosus*. Population differentiation was assessed using exact tests as implemented in GENEPOP (Raymond & Rousset 1995a, 1995b) among populations from the North Sea (Lysekil, common morph, salinity 25 psu) and from three areas of the Baltic (Öland, common morph, 7 psu; Öregrund, common and dwarf morph, 5 psu; Umeå, dwarf morph, 4 psu) (Fig. 1a). In Öregrund, dwarf and common morphs formed a mixed population; no spatial segregation of the two morphs was observed in the area where samples were collected. All samples, including the sympatric one from Öregrund, were collected from an area of about 2 m². The populations from Öregrund (common and dwarf) and Umeå were collected after the peak of the reproductive period with only 8, 10 and 18% of the individuals, respectively, still carrying receptacles. However, observations from other years indicate that main part of the individuals in these areas produce receptacles in both morphs. The populations from Öland and Lysekil were all sampled during the reproductive period and consisted of 100% sexually mature individuals.

Cavalli-Sforza and Edwards' chord distances based on allele frequencies were used to construct the neighbour-joining tree with PHYLIP (Felsenstein 1993). We found support for the tree nodes by bootstrapping the allele frequency matrix (1000 iterations). The expected frequency of a multilocus genotype, P_{gen} , and the probability of observing n individuals of each multilocus genotype by chance in a sample of N individuals, P_{sex} , were calculated according to Ivey & Richards (2001).

Re-attachment experiment

Fragments of thallus from the dwarf and the common morphs of *F. vesiculosus* were placed on granite discs and allowed to re-attach under laboratory conditions at 10 °C and 60 $\mu\text{Mols}^{-2} \text{m}^{-1}$ PFD in untreated local brackish water (5 psu). The treatments were: excised adventitious branches (5–10 mm), apical cuttings (5 mm), and scrapes of thallus wings (1–3 mm), adding > 20 fragments per replicate (mix from 15 individuals, $n = 4$). The rates of re-attachment were calculated after 7 weeks by rinsing the granite discs with water and counting the proportion of attached fragments.

Results and discussion

All five microsatellite loci were highly polymorphic with five to 10 alleles per locus. We found high differentiation in allele and genotype frequencies between sympatric populations of the common and dwarf morph at Öregrund, at all five microsatellite loci studied (exact probability tests, $P < 0.001$ in all comparisons). This suggested that there is a barrier to gene flow between the dwarf and the normal morph of *Fucus vesiculosus*, and therefore, we consider them separately in all further analyses. Moreover, the two dwarf populations were genetically differentiated from the five populations of common *F. vesiculosus* (Fig. 1b). The two dwarf populations were, however, only slightly differentiated from each other, although separated by 400 km (differences are nonsignificant at all loci considering all individuals, but significant at locus L20, $0.01 < P < 0.05$, after excluding multiply sampled clones).

Unexpectedly, we observed numerous identical multilocus genotypes in both samples of the dwarf morph. Thus, 35 out of 48 sampled individuals from one of the populations (Umeå, D1 in Fig. 1b) and 40 out of 48 individuals from the other population (Öregrund, DM in Fig. 1b) had identical genotypes at all five loci, being heterozygous at

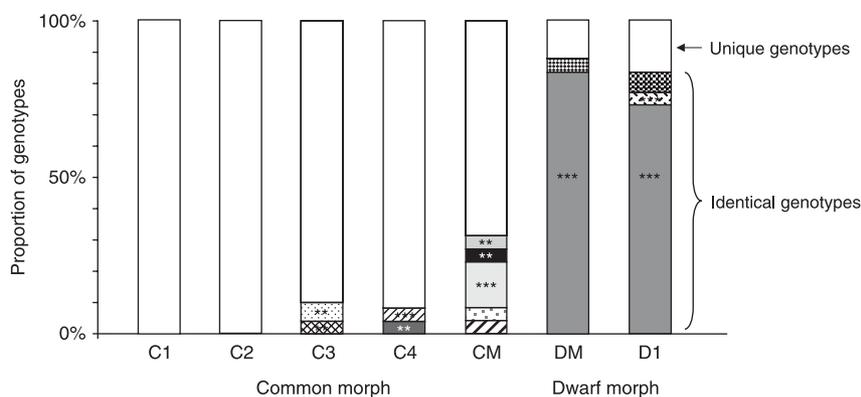


Fig. 2 Proportion of genotypes in *Fucus vesiculosus* populations. Differently patterned portions of the bars indicate proportion of individuals with identical multilocus genotype profiles, most of which correspond to clones, but some may be identical by chance. Genotypes denoted with the same pattern are identical across populations. White portions of the bars represent unique genotypes probably produced by sexual reproduction. Levels of significance for P_{sex} , the probability to obtain observed numbers of identical multilocus genotypes by chance, are indicated as following: *, $0.01 < P < 0.05$, **, $0.001 < P < 0.01$, ***, $P < 0.001$. The sample sizes are 48 individuals for DM, CM, and D1 populations and 50 individuals for C1–C4, in total 344 individuals. A total of 250 distinct multilocus genotypes were found among these. Sample designations as in Fig. 1.

four of them. It is extremely improbable to observe such numbers of identical genotypes in sexually reproducing, randomly mating populations ($P_{sex} < 10^{-10}$ in both populations) and we conclude that these are all members of the same clone. Indeed, even nonrandom mating could not account for the observed pattern. If we assume that only a part of all plants participate in reproduction and estimates of allele frequencies for that subpopulation are unknown, with four heterozygous loci in the multilocus genotype the maximum attainable frequency of that genotype, P_{genr} is 0.0625. Expanding the binomial term $(0.0625 + 0.9375)^{48}$ we find that it is unlikely to observe seven or more identical multilocus genotypes in a sample of 48 individuals ($P_{sex} < 0.05$), let alone the 35 or 40 ($P_{sex} < 1 \times 10^{-10}$). Besides this dominant clone, we found occasional individuals of identical multilocus genotypes indicating one to three additional clones in the dwarf morph (Fig. 2). We also found evidence of seven to nine clones present in the Baltic populations of the common morph, with a tendency of increasing clonality with decreasing salinity (Fig. 2).

Clones might constitute a significant part of populations in, for example, the seagrass *Zostera marina* (Olsen *et al.* 2004) and in particular red algae (Gabrielsen *et al.* 2002). It is also known that detached fragments of fucoid algae may continue to grow (Lee 1989). However, this is the first report of the occurrence of clones among attached fucoids. Until now it was believed that reproduction of attached individuals in this group was entirely sexual (Fig. 3a); no vegetative spores are known and excised parts of fronds are thought to be unable to re-attach. Moreover, a previous comprehensive study employing microsatellites did not find any evidence for clonality in a closely related species (*Fucus serratus*) over its whole species range (Coyer *et al.* 2003). Our own population surveys using microsatellites also fail

to uncover indications of asexual reproduction in attached populations of *F. vesiculosus* outside Baltic including populations in estuarine environments (we have unpublished data from 66 populations over 20 regions ranging from Morocco to North Sea and Iceland).

In order to establish a mechanism for asexual reproduction in *F. vesiculosus*, we tested whether and which detached fragments of the thallus might have the capacity to re-attach. Adventitious branches were the only type of fragment that produced thallus-like shoots that re-attached by regenerating rhizoids from the wounded basal part (Fig. 3b–e) while apical cuttings and scrapes of thallus failed to produce rhizoids. The rate of re-attachment was particularly high in the dwarf morph, but some adventitious branches of the common morph re-attached as well (Fig. 3b).

It is considered that adventitious branches are formed in response to wounding (Moss 1964; Van Alstyne 1989). However, they are increasingly common in the northern Baltic Sea, where they are also conceived as a stress response to low salinity (Waern 1952). Adventitious branches are known to detach and give rise to loose-lying algae in several fucoid species (Lee 1989). Now we show that these structures also have the potential for being vegetative propagules establishing attached algae on rocky shores. A sublittoral growth of *F. vesiculosus* and occasional extended periods of calm water in the atidal Baltic Sea are likely to increase the probability for successful re-attachment of vegetative propagules in the field.

Three observations are intriguing in the case of asexual reproduction in Baltic populations of *F. vesiculosus*: the extent of clonal development, its genetic predisposition (indicated by different degrees of clonality in the two morphs), and its association with physiological stress experienced by the algae in a marginal habitat. Two main

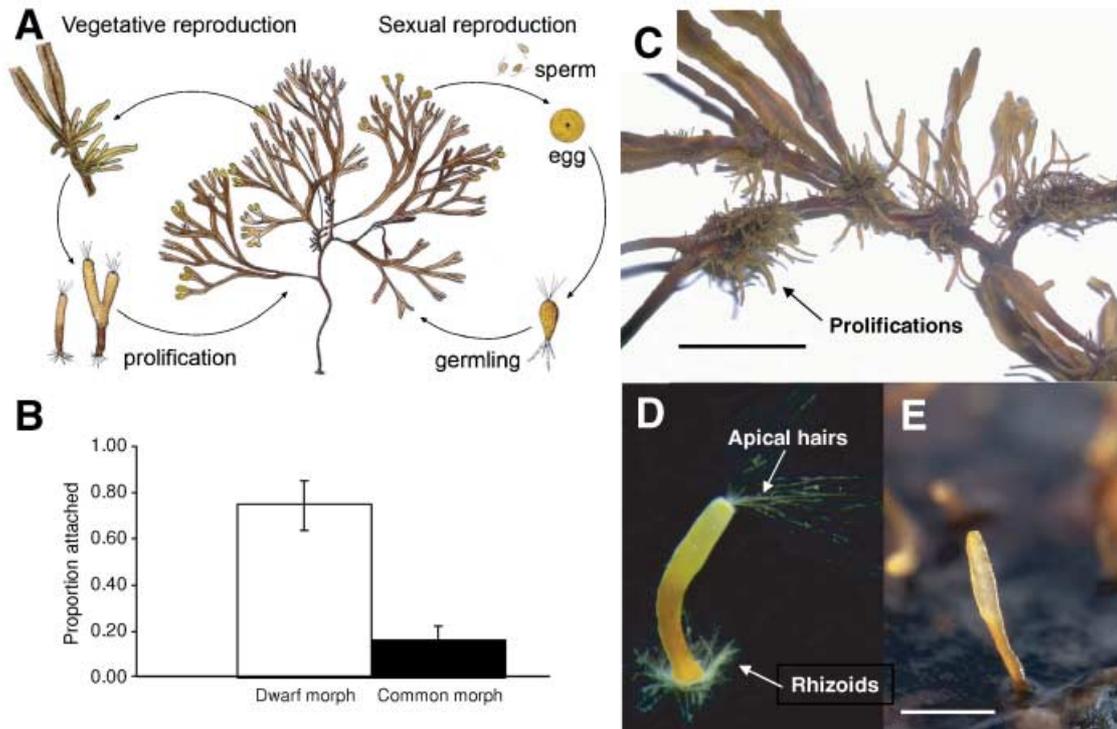


Fig. 3 Asexual reproduction in *Fucus vesiculosus*. (A) The Life cycle of *Fucus* is diplontic: the adult thallus is diploid, and the haploid phase consists of unicellular gametes produced by meiosis and multiplied by mitosis in the gametangia. *Fucus vesiculosus* is dioecious, with male and female gametes produced by separate, unisexual, individuals. Vegetative generation of clonal attached algae reported here is a novel finding in *Fucus*. (B) Proportion of adventitious branches re-attached to substratum. Error bars indicate maximum and minimum values observed in four replicates per each morph. (C, D, E) Adventitious branches on an adult *F. vesiculosus* (C) and attached to substratum (D, E). Developed rhizoids and apical hairs indicate fully functional organism after 7 weeks of experiment (D). Scale bars, 2 cm (C), 2 mm (D, E).

models have been suggested to explain 'geographical parthenogenesis', that is, patterns of asexual reproduction associated with marginal environments. The first simply predicts that asexual reproduction is promoted in environments in which sexual reproduction is difficult or impossible (Stalker 1956; Kramer & Templeton 2001). The second model emphasizes movements of individuals from areas of good to poor conditions (a 'source-sink' relationship) favouring fixation of genotypes that survive marginal conditions through asexual reproduction (Peck *et al.* 1998). Marine organisms encounter major problems to persist in low-salinity environments such as the Baltic Sea, but populations of Baltic fucoids are dense and healthy, lending minor support for the source-sink model for this specific species. Sexual reproduction, however, appears to be a main difficulty in brackish conditions, particularly for externally fertilizing organisms like fucoid algae. In *F. vesiculosus*, low salinity reduces longevity and motility of gametes (Serrão *et al.* 1996). Indeed, at the northern limit of *F. vesiculosus* in the Baltic, gametes are rarely released, fertilization success can be very low, and even when eggs are fertilized they suffer from significant polyspermy (penetration of the egg by more than one sperm, which is lethal)

(Serrão *et al.* 1999). In such conditions asexual reproduction should be strongly favoured by natural selection, as suggested by the model of Stalker (1956). Moreover, vegetative propagation of clones is a plausible explanation to the observation made by Serrão *et al.* (1999) that the populations of dwarf morph along the northern Swedish coast are strongly female-biased (> 80% female individuals). The reason clonality is not observed in other estuarine environments in, for example, Portugal and France (E. Serrão, personal communication) might be that here the algae are mainly out of the water during the low tide periods when salinity is low, while at high tide the salinity increases. In the Baltic Sea, the fucoids live permanently submerged in low-salinity waters. Thus, the use of adventitious branches as vegetative propagules, seems an innovative evolutionary step strongly selected for in the presence of physiological barriers to sexual reproduction in a truly extreme marine habitat.

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