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As tides go by

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Chapter 7

Summary

The variety of life histories found in seaweeds has fascinated evolutionary biologist, ecologists and phycologists for the past century. This evolutionary diversity ranges from the familiar monophasic diploid forms to bi- and tri-phasic alternations of generations. Many separately described species were later found to be the alternate, free-living haploid and free-living diploid phases of a single species. In addition, one or both phases may reproduce clonally thus further complicating the dynamics of their life histories. The presence of both haploid and diploid free-living forms raises questions about the evolutionary maintenance of these life cycles as well as their ecological significance. What advantages can such complicated life histories confer?

A few studies have explored alternation of generations in heteromorphic species—mainly in a community ecology context. Differential grazer resistance has been demonstrated between encrusting vs. frondose forms; some crustose forms have been shown to be competitively superior space holders to the upright form; and differences in morphology have been associated with differential photosynthesis performance. Only one isomorphic species has been investigated. Gametophytes and sporophytes were identified by seasonal reproductive structures. Subsequent lab studies revealed faster growth rates in the haploid phases, but higher tolerance to Pb for the diploid phase. Studies of alternation of generations in a population genetics context are still in their infancy mainly because earlier generation allozyme markers were not polymorphic enough and/or technically problematic. Only with the recent addition of microsatellites (offering at least a 10-fold increase in resolution) has this situation begun to change.

The central aim of this thesis was, therefore, to investigate population genetic structure at different spatial scales in a seaweed characterized by an isomorphic, haplodiaplontic life history and a putatively poor dispersal capacity.

The filamentous green seaweed *Cladophoropsis membranacea* grows on rocky coastlines and coral reefs in wave-swept of high-surge zones. It is one of the main, space holding species in these communities and can become transiently dominant. Haploid and diploid plants cannot be distinguished in the field without genetic markers. Asexual reproduction is supposed to be characteristic of both phases. A number of questions were raised in the Introduction that were subsequently investigated in the various chapters. Here the first set of key questions are restated:

- Are local populations of *C. membranacea* sequentially dominated by the haploid or the diploid phases of the life history, or do both phases co-exist throughout the year?

- To what extent does asexual reproduction augment one or both phases?
- Are local populations of mixed haploid and diploid phases in Hardy-Weinberg Equilibrium?
- Are there differences in allele frequencies between haploids and diploids living in "apparently" the same habitat?
- How far is short-distance dispersal of spores and/or zygotes?
- Are they related to each other?

Small-scale population structure was investigated at three different locations on Tenerife and Gran Canaria (**Chapter 3**). At all sites haploid, diploid and clonal thalli were found but haploid thalli were always dominant (from 2:1 to 10:1). The within-site clonal diversity was also very high for the haploid phase; in contrast, only a single diploid clone was found. Haploid clones accounted for 25-60% of the individuals at most sites. There were no significant differences in allele frequencies between phases although significant positive departures from Hardy-Weinberg Equilibrium were found. Small patches of related individuals may account for the heterozygote deficiency. Linkage disequilibrium was also associated with haploid plants but attributed to drift. Both of these results are consistent with local patches in which spatial autocorrelation analysis of dispersal distances were ≈ 1 m (and rarely further than 5 m).

The above analyses were made during only one time in the year (January). Therefore, the dominance of the haploid phase may still reflect seasonal shifts in the relative frequencies of haploids and diploids. So, do diploids seasonally dominate? The answer is simply unknown. An alternative hypothesis is that the haploid phase is locally superior and competitively dominant. Testing for fitness differences in the field is not feasible but definitely possible in the laboratory. In any case, such a strategy would be theoretically favored in disturbed environments. Although sexual reproduction appears to be infrequent in *C. membranacea*, it is clearly sufficient enough to maintain both life history phases. This supports theoretical modeling in which haploid-diploid life histories comprise an evolutionary stable strategy.

The next set of key questions included:

- How are populations of *C. membranacea* differentiated within and between islands?
- What is the minimum spatial scale at which it can be detected?

Limited dispersal at the level of a few meters leads to the prediction of strong population differentiation even at very small spatial scales. Significant F_{ST} values were found, for example, at scales of as low as 1.3 to 5 km, which follows a spore retention model.

In order to explore the scope of the differentiation further, isolation by distance was tested under a stepping stone model (**Chapter 4**). Approximately 75% of the variation occurred within sites and 25% between sites separated by 1-125 km on an island. Thus, almost all of the variation was within islands; only 6% of the varia-

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tion being accounted for between islands ($\approx 100\text{-}300$ km apart). Mantel tests were significant for Gran Canaria and for Tenerife but not for Fuerteventura. Mantel tests were not significant among the Canary Islands. Only when the comparison was extended to include the Cape Verde Islands, was any significance observed. How can this seemingly paradoxical result be explained, i.e., strong differentiation and isolation by distance at small distances and weak to absent isolation by distance at a larger distance? The short answer is non-equilibrium conditions that favor genetic drift over gene flow in relation to spatial scale.

The lack of equilibrium in the intertidal surge zone is no surprise but what about at the scales of within and between islands or further out? It is also no surprise that short-distance dispersal of spores, gametes and small propagules are extremely limited (probably in a retention zone), while long-distance dispersal by floating or rafted thalli on debris—though rare—does occur. Theory predicts that poor dispersal capacity will lead to strong differentiation—which is observed up to a point. What's being overlooked? Non-equilibrium conditions at the larger geographic scale are particularly relevant in the Canary Islands which straddles a crucial biogeographic temperature boundary and which has been subject to oscillation in sea surface temperature on a regular basis since the last glacial maximum (ca. 20,000–18,000 years ago) including the little ice age (ca. 700–150 years ago). Present-day “fuzziness” reflects past selection regimes and genetic drift that are working faster than long-distance dispersal and gene flow.

The final set of questions asked were:

- To what extent are the Tethyan vicariance models put forth by Kooistra *et al.* (1992) and Pakker *et al.* (1994) supported or refuted by the new data from the Atlantic Ocean?
- Is there any new evidence for the presence of cryptic species?

The original sampling was confined to only a few isolates and a few ITS sequences. One of the goals was thus to reevaluate the vicariance hypothesis using >1,600 individuals and the microsatellite loci (**Chapter 5**). It was soon discovered that many of the samples could not be amplified for any of the microsatellite loci. Since null alleles had not presented a particular problem in the Canary Island samples, the sudden locus drop-out signaled the possibility of cryptic species. Moreover, the distribution of amplifiable and non-amplifiable loci was not strictly geographically related. Within the Canaries, Cape Verdes and Caribbean, examples were found of both. This led to a new ITS sequencing effort involving samples from 30 locations. Three cryptic species were identified which caused a complete reevaluation of the 1992–1994 work based on the combined data from ITS sequence divergences, differential microsatellite amplification and knowledge about thermal ecotypes. Rooting the tree with Pacific isolates, resulted in a monophyletic clade that included the Central Canary Islands, Cape Verde and Bonaire; a second clade stretching across the SE Caribbean to the Cape Verde Islands and African coast but excluding the Canary Islands; and finally a NE Canary Island group that include the Mediterranean and Red Sea. The Tethyan vicariance hypothesis was not falsified though our

understanding of the complexities is substantially greater; and the correlation with temperature ecotypes also remains well supported. The detection of overlapping biogeography distribution highlights the importance of ecotypic differentiation and the importance of shifting sea surface isotherms that have driven periodic extinction and recolonization of the Canary Islands—a crossroads for marine floral exchange.

Concluding Remarks and Prospects

Ecological

Genetic markers are now available for an increasing number of algal species exhibiting different life histories, living in different habitats and interacting with different community members. Therefore, the opportunity to quantitatively address process-based questions about dispersal, selection and adaptation are now possible for seaweeds; as are experimental questions related to fitness differences, competition and facilitation.

At present it is not known whether *C. membranacea* is a perennial alga that dies back each season to regenerate from microscopic, over-wintering thalli/propagules. Future studies must add this annual component as well as a seasonal one. The importance of seasonality (or lack thereof), persistence vs. new recruitment (possibly from spore banks) and competition/facilitation between phases can now be assessed; as can fitness differences between haploid and diploid clones.

Technical

The development of genetic markers—in this case microsatellites (**Chapter 2**)—in algae is still a challenge that should not be underestimated. Although the technology and collective experience have greatly advanced since the start back in 1994 (not too much known about enriched libraries; no automated sequencing; little collective experience outside of a few model systems), it is clear that algal genomes are different. Microsatellite density seems to be lower and less polymorphic—and not just in *C. membranacea*. Why this should be so remains a mystery. In **Chapter 6**, a number of “strange encounters” during the development of microsatellite loci in *C. membranacea* were discussed. Nearly half of the loci were associated with other repetitive sequences. Whether this is a generalised phenomenon remains uncertain but other laboratories who have used different protocols and worked with other algal species (six as of now) have encountered similar findings and difficulties. The recommendation is: use multiple enriched libraries, sequence many clones from the outset (it will save you time, money and headache later), align and check your clone sequences as you go along.

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