GENETIC VARIATION WITHIN TWO PULMONATE LIMPET SPECIES, *SIPHONARIA CAPENSIS* AND *S. SERRATA* ALONG THE SOUTH AFRICAN COAST

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ABSTRACT

Dispersal rates are known to play a fundamental role in establishing the genetic structure found within a species. Dispersal in marine benthic invertebrates is largely dependent on life history strategy. The presence of pelagic larvae will potentially result in high dispersal within a species, while direct development limits dispersal. This study used two intertidal pulmonate limpet species to investigate the relationship between dispersal potential and genetic structure of populations. *Siphonaria capensis* produces benthic egg masses, which release pelagic larvae. These float in ocean currents before settling and metamorphosing into adults. *S. serrata* reproduces by direct development. The larvae hatch as completely metamorphosed juveniles after developing in an egg case not far from the parent. The high dispersal capacity of *S. capensis* pelagic larvae should result in high levels of within-population variation since individuals within a particular population may originate from several different populations. This should lead to high levels of gene flow along the coast and to low genetic differentiation among populations. On the other hand, the low dispersal potential of *S. serrata* should cause low levels of genetic variation within populations, as relatives will remain close to one another. Low levels of gene flow and high genetic differentiation among populations are expected in this species. In dealing with the relationship between life history strategy and population genetic structure in these two species, a series of indirect techniques was utilised. Multivariate analyses of the morphological variation within each species showed that variation in size was similar between the two species and largely governed by environmental factors. Variation in shell shape was largely under genetic control and supported the predictions made for each species. Variation

in total proteins was relatively uninformative in terms of examining the differences between the two species. Allozyme analysis and mtDNA sequencing clearly showed differences between the species in terms of their population genetic structure. High levels of gene flow were found within *S. capensis*. This was strongly influenced by ocean currents, with the close inshore Agulhas current along the Transkei coast contributing to high levels of dispersal and hence gene flow. Low levels of gene flow occurred within *S. serrata*, resulting in low within-population variation and high among-population differentiation.

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CHAPTER 1: General Introduction

> "These forms which possess in some considerable degree the character of species, but which are so closely similar to some other forms, or are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us." (Darwin, 1859, p47 cited in Endler, 1977).

At the very base of diversity in nature lies variation in individuals. Gene flow lies at the heart of both macroevolutionary processes, such as speciation, biodiversity, systematics, and microevolutionary processes, such as population genetics - the main topic of this thesis. Traditionally, the disciplines associated with variation in population genetic structure and those associated with variation in phylogenetics and macroevolutionary processes have remained separate (Avise *et al.*, 1987). Recently, however phylogeography (based mainly on mitochondrial DNA analysis) has provided a bridge between the two areas of study (Avise, 1998; Bermingham and Moritz, 1998). For the first time, in 1943, Mayr emphasised the importance of gene flow in determining population genetic structure (Slatkin, 1985). Gene flow is defined as the movement of genes and/or gene complexes in populations (Endler, 1977; Neigel, 1987; Slatkin, 1987; Mitton *et al.*, 1989) as well as their establishment in allochthonous gene pools (Endler, 1977). Movement of genes is achieved through the movement of extranuclear fragments (such as mitochondria and plasmids), gametes, zygotes, individuals or groups of individuals and possibly even through extinction and recolonisation of entire populations (Schaeffer *et al.*, 1985; Slatkin, 1985 and 1987). However, the process of extinction and recolonisation is considered to increase gene

flow only under very particular ecological conditions and is not considered a common event on a large physical scale (Wade and McCauley, 1988). A number of different views exist on the importance of gene flow. The two extreme views can be summarised as follows:

1. gene flow is common and even a low level of gene flow among populations in different areas of a species' range will effectively unify a species (Slatkin, 1981). A continually high level of gene flow will not allow for the establishment of a particular combination of genes within a population (Berglund and Lagercrantz, 1983; Slatkin, 1985). Ultimately, gene exchange will prevent localised adaptation (Scheltema, 1971; Jackson and Pounds, 1979) and so any effect of selection will only occur within a single generation (Johnson and Black, 1984a).

2. gene flow is uncommon (Ehrlich and Raven, 1969) and natural selection and adaptation act more or less independently in each area of the species' range (Slatkin, 1981). Low levels of gene flow will give well-adapted combinations of genes the opportunity to become fixed. Occasional colonisations will then spread these gene combinations to other populations (Berglund and Lagercrantz, 1983; Slatkin, 1985).

The level of gene flow within a species is usually linked to dispersal potential (Scheltema, 1971 and 1975; Ward, 1990; Hurst and Skibinski, 1995; Palumbi, 1995; Bohonak, 1999). Most marine intertidal benthic invertebrates are assumed to move very short distances as adults (Marcus, 1977; Knowlton and Keller, 1986), unless "rafting" is achieved by the adults (Martel and Chia, 1991; Grant and da Silva-Tatley, 1997). To a large degree, the extent of dispersal (and hence gene flow) is strongly influenced by the degree of larval dispersal. This, in turn depends largely on the mode of larval development (Burton, 1983; Janson, 1985a; Perron and Kohn, 1985;

Strathmann, 1985; Grant and Utter, 1987; Russo *et al.*, 1994; Todd *et al.*, 1994; Hellberg, 1995; Bohonak, 1999; Jones *et al.*, 1999; Pechenik, 1999; Kyle and Boulding, 2000). A broad range of developmental modes exists among marine invertebrates. The most common types identified include: planktotrophic development; lecithotropic pelagic development; demersal larval development; and direct development (Thorson, 1950; Mileikovsky, 1971; Vance, 1973; Berry, 1977; Grahame and Branch, 1985). These various developmental strategies can be viewed from a number of different perspectives (e.g. Mileikovsky, 1971; Pechenik, 1979; Tatarenkov and Johannesson, 1994; Pechenik, 1999). One view is to divide them into groups based on their capacity for dispersal (Tatarenkov and Johannesson, 1994):

1. **high dispersal potential** -through planktotrophic larvae which disperse in the pelagic zone for weeks to months
2. **moderately high dispersal potential** -through lecithotrophic larvae which disperse in the pelagic zone for hours to days
3. **low dispersal potential** -direct development with no free larvae.

While these different developmental modes should have a strong influence on gene flow within a species, simply having the potential for high dispersal does not automatically result in high levels of gene flow (Hedgecock, 1982; Palumbi, 1995). The length of larval life in the water column is influenced and often limited by factors such as temperature, food availability and settlement responses. Post-settlement mortality (Mallet *et al.*, 1985; Prince *et al.*, 1987; Miller, 1998) and larval behaviour may also reduce the effect of dispersal on populations (Marcus, 1977; Davies and Butler, 1989; Bingham and Young, 1991; Bertness and Gaines, 1993; Todd *et al.*, 1998). Dispersal of larvae (and any adults which may disperse by rafting) is also

affected by physical parameters, such as ocean currents and coastal topography (Vermeij, 1987; Shepherd *et al.*, 1992; Bertness and Gaines, 1993; Hellberg, 1998). Regardless of the amount of larval dispersal which occurs, successful gene flow requires survival (Jurss *et al.*, 1999) and more importantly successful breeding of migrants in their new populations (Scheltema, 1975; Endler, 1977; Skelton, 1993). Once successful gene flow occurs among populations of a single species, it is likely to result in genetic homogeneity among populations (Slatkin, 1987; Bohonak, 1999). Homogeneity can thus exist over great distances, commensurate with the distances over which large numbers of larvae can disperse.

While gene flow plays an important role in population genetic structure, it is not the only contributing factor. In fact, genetic variation results from a balance between forces causing genetic homogeneity and those producing local genetic differentiation across the entire species range (Slatkin, 1987; Skelton, 1993; Jollivet *et al.*, 1995; McQuaid, 1996b; Bohonak, 1999). While gene flow usually prevents divergence of populations (Ehrlich and Raven, 1969; Slatkin, 1987; Grant and da Silva-Tatley, 1997), mutation, genetic drift and natural selection all favour adaptation to the local habitat (Newkirk and Doyle, 1979; Slatkin, 1987). These factors, in turn, lead to increased genetic differentiation among local populations (Arnaud and Daguzan, 1999). The relative importance of the mechanisms responsible for genetic variation is a highly controversial issue, on which there is still little consensus (Tatarenkov and Johannesson, 1994; Johannesson *et al*, 1995). However, of the mechanisms affecting variation in populations, it has been suggested that apart from gene flow, natural selection or genetic drift are the most important (Scheltema, 1975; Johannesson and Tatarenkov, 1997; Bohonak, 1999).

Darwin first described natural selection in the 1850's as "the preferential survival of individuals with profitable variations" (Skelton, 1993 p.5). Since then, the role of natural selection and variation in the greater scheme of biological evolution has been discussed and debated at length (Ehrlich and Raven, 1969; Ohta, 1974; Skelton, 1993). In this study, natural selection is considered as one of the processes antagonistic to gene flow because it may cause high genetic variation within a species. As previously stated, there is still considerable debate over the power of natural selection on population genetic structure. Ehrlich and Raven (1969), Jackson and Pounds (1979), Newkirk and Doyle (1979), Johnson and Black (1982), Noy *et al.* (1987), Johannesson *et al.* (1990 and 1995), Grahame *et al.* (1992) and Johannesson and Tatarenkov (1997) all emphasise that natural selection is important in controlling genetic differentiation, particularly on a small scale (over metres). However, other authors argue strongly that selection is not the cause of genetic differentiation found among natural populations. Instead, they suggest that such differentiation is caused by founder effects, random genetic drift and possibly genetic bottlenecks (Njiokou, *et al.*, 1994; Jollivet *et al.*, 1995; Todd *et al.*, 1998).

Genetic drift is the unpredictable change in gene frequency due to finite population size (Slatkin, 1987) and, like gene flow, it has the same average effect on all nuclear genes. In completely isolated populations, genetic drift tends to fix different alleles in different local populations, thereby increasing genetic differentiation among populations (Slatkin, 1987). There is much debate as to the level at which genetic drift operates. Some researchers consider that natural selection will over-power genetic drift and will be responsible for most of the genetic variation found at a local level

(Bucklin and Marcus, 1985), particularly on a small scale (Black and Johnson, 1981; Slatkin, 1987; Janson and Ward, 1984; Johannesson and Johannesson, 1989; Johannesson *et al.*, 1990). Others consider genetic drift to be far more important in fixing alleles in local populations, particularly on a larger scale (Skibinski *et al.*, 1983; Todd *et al.*, 1998).

The processes discussed all operate on populations and it is important to attempt to define precisely the term population. This term is often loosely used and holds a variety of meanings, depending upon researchers' interpretations. Endler (1977) stated that "in the general sense, a population is any group of organisms of a single species". Begon (1990) went further to define a population as "a group of individuals of one species in an area, though the size and nature of the area is defined, often arbitrarily, for the purposes of the study being undertaken". Over the years, various mathematical models have been constructed to predict geographic population structure within a species. Broadly, two main types of population structure can be presumed; a population may consist of discrete subpopulations, or alternatively a population may consist of continuously distributed individuals (Slatkin, 1985). Generally, it is assumed that marine organisms (like many of their terrestrial counterparts) are not uniformly distributed but rather are typically restricted on a local scale to patches of favourable habitat (Burton, 1983). So most populations appear to be composed of discrete "subpopulations". In turn, these discrete sub-populations are envisaged to conform to one of two models, either the island model or the stepping-stone model (Neigel, 1997). Migration or gene flow is considered to be equally likely between all populations in the case of the island model, but is limited to neighbouring populations in the stepping-stone model (Hartl and Clark, 1989). When working with populations

along a coastline, which is essentially a linear habitat, it seems more appropriate to apply the stepping-stone model (Janson and Ward, 1984; Slatkin, 1985; Neigel, 1987). Hence a population in this study is taken to be any group of individuals, belonging to a single species, from a single location. It is assumed that if populations are linked in any way that they will follow the stepping-stone model.

Population genetic structure and gene flow can be examined using two groups of methods: direct and indirect methods. Direct methods usually involve some form of mark and recapture technique. This indicates the level of gene flow occurring under present conditions rather than inferring average levels of gene flow (Slatkin, 1985; Bohonak, 1999). This type of method may lead to either underestimates, through unmarked individuals moving, or to great overestimates as marked individuals may fail to breed (Slatkin, 1981). Although some studies have successfully tracked larvae dispersing in ocean currents (Beckley and Hewitson, 1994; Palumbi, 1995; Beckley, 1997), population genetic studies in the marine environment, generally do not lend themselves well to direct measures of gene flow (Knowlton and Keller, 1986; Liu *et al.*, 1991). Tracking planktonic larvae is, in fact, often considered virtually impossible (Mitton *et al.*, 1989; Todd, *et al.*, 1998). Indirect measurements of dispersal, and hence gene flow, require greater assumptions than direct methods but they can detect occasional or unpredictable changes in gene flow and give an indication of genetic structure averaged over thousands of generations (Bohonak, 1999). Molecular biological techniques, as indirect measures of gene flow, have provided detailed information about genetic structure of natural populations and can be used to calculate levels of gene flow accurately (Slatkin, 1987; Palumbi, 1995; Avise, 1998). The development of these techniques has resulted in a large number of studies on

population genetics on a wide variety of animals, mostly marine invertebrates (Bohonak, 1999). There are limitations to indirect methods, but if sufficient loci are analysed and if there is consistency in the estimations using different molecular techniques, then there can be reasonable confidence in the findings (Slatkin, 1985; Bohonak, 1999). The use of both protein and DNA markers in examining population biology can be enormously useful as often different markers appear to reflect the predominance of different evolutionary forces (Mitton, 1994; Hillis and Moritz, 1990).

In this study, various techniques have been used to examine the relationship between life history strategy and population genetic structure in two species of *Siphonaria* along the coast of South Africa. These pulmonate limpets are the most primitive of the gastropods (Barnes, 1987). The higher classification of the pulmonates is far from settled, though at present these false limpets fall into the Order Basommatophora (Hodgson, 1999). Within this order, over 60 species have been described and marine pulmonate limpets make up the family Siphonariidae. There is still debate as to how many genera occur within the Siphonariidae. The two species used in this study fall into the genus *Siphonaria*. Prior to 1946 it was thought that this genus included 13 species along the South African coast (Allanson, 1959) but it is now accepted that nine South African species exist (Chambers and McQuaid, 1994a). This study concentrates on two such species, *Siphonaria capensis* and *S. serrata*. Briefly, these two species are common intertidal herbivores, which show hermaphroditism, internal fertilisation and the production of gelatinous benthic egg masses (Allanson, 1959; Chambers and McQuaid, 1994a). The striking difference between these two species lies in their differing developmental modes.

In dealing with the relationship between life history strategy and population genetic structure in *S. capensis* and *S. serrata* this thesis is set up as follows. The second chapter serves to familiarise the reader with the two species and provides details of the study area, including the hydrography around the coast of South Africa. Following this is a short chapter on the advantages and disadvantages of various indirect techniques available for examining genetic variation. The thesis then moves through a series of techniques, which were used to investigate the hypothetical link between larval type and genetic structure of *S. capensis* and *S. serrata*. Firstly, variation in morphology was examined. This was carried out to establish if multivariate analysis of shell size and shape could be a reliable indicator of genetic variation. Variation in total soluble proteins was then examined. This is a simple and inexpensive technique and it may prove useful in population studies if it provides reliable data. Allozyme analysis is then reported on. This is a very widely utilised technique. Finally, mtDNA sequencing was used to examine population variation in *S. capensis* and *S. serrata*. This is generally considered an expensive, very specialised technique but it examines the heart of genetic variation by revealing the nucleotide sequence of a particular gene. Together, these different techniques provide a thorough and extensive picture of genetic variation in both *S. capensis* and *S. serrata* along the coast of South Africa, as shown in the final discussion (Chapter 8).

CHAPTER 2: Study organisms & study sites

2.1 STUDY ORGANISMS

The Siphonariidae are successful intertidal limpets. They are considered the most primitive of the pulmonates and are evolutionarily distinct from the other primitive prosobranch limpets (Allanson, 1959; Chambers and McQuaid, 1994a; Hodgson, 1999). Their origins are unclear (Barnes, 1987; Chambers and McQuaid, 1994b) but the evidence suggests that these pulmonate or "false" limpets have a marine ancestry rather than a terrestrial ancestor that re-invaded the marine environment (Marshall and McQuaid, 1991; Hodgson, 1999). Evidence for this view is given by the fact that planktotrophic development is considered to be primitive and unlikely to be re-acquired once lost (Hodgson, 1999; Pechenik, 1999). Over 60 species have been recorded in the family Siphonariidae, but there is a continuing debate about how many genera should be recognised. At present, four genera are recognised: *Siphonaria, Williamia, Kerguelenella* and *Benhamina* (Hodgson, 1999). Within the genus *Siphonaria*, there are nine valid South African species (Chambers and McQuaid, 1994a).

Generally, there is a greater diversity of *Siphonaria* species in warm temperate and subtropical waters than in cool waters. This is clearly demonstrated along the South African coast. Only three species are found in cool temperate waters along the west

coast, while seven species are found along the subtropical east coast (Hodgson, 1999). The success of these abundant limpets, especially in warmer coastal areas, is due to important physiological and behavioural adaptations (Hodgson, 1999). Physiological adaptations include: rapid re-hydration rates; efficient air and water respiration through the possession of both a gill and a pulmonary cavity; anaerobiosis; and tolerance to a range of salinities (Marshall and McQuaid, 1989; Hodgson, 1999). In combination with these physiological abilities, foraging behaviours, the use of home scars and clustering help to prevent extreme desiccation (Branch, 1981; Hodgson, 1999).

All siphonariid limpets are hermaphroditic with internal fertilisation (Allanson, 1959; Chambers and McQuaid, 1994a; 1994b). They produce gelatinous egg masses, which are attached to the substratum but vary in shape among species. Modes of development range from long-lived planktotrophic larvae to the hatching of completely metamorphosed juveniles (Chambers and McQuaid, 1994a). Direct developers produce egg masses with a low surface area to volume ratio and a thick outer layer. These egg masses spend up to four weeks on the shore before hatching (Chambers and McQuaid, 1994a). Planktotrophic developers produce egg masses which have a high surface area to volume ratio and these only remain on the shore for up to a week before the larvae are released into the sea (Chambers and McQuaid, 1994a). Further development of the young varies greatly among species.

Ideally, population genetic studies should be carried out on closely related species, between which the greatest difference is their mode of larval development (Hellberg, 1996; Bohonak, 1999; Kyle and Boulding, 2000; Collin, 2001). Thus, *Siphonaria capensis* and *S. serrata* were selected as ideal study organisms, as they represent two

extremes of larval development. In addition, siphonariids are not palatable to predators or humans, largely because of the copious quantities of mucus produced, which contains polypropionates (Hodgson, 1999). People living in the coastal areas of South Africa, particularly along the Transkei coast, exert extremely high predation pressure on animals, so the fact that *S. capensis* and *S. serrata* are unpalatable ensures their presence at all locations along the coast and eliminates the possibility of selection through human predation.

Siphonaria capensis produces egg masses which release planktotrophic larvae. These float in the ocean currents for at least one month before settling and metamorphosing into adults (Chambers and McQuaid, 1994b). Such larvae should result in high levels of dispersal along the coast. In turn, this is expected to result in high genetic differentiation within populations as individuals within any particular population may originate from a number of different parent populations. At the same time, the high dispersal potential of *S. capensis* should cause high levels of gene flow along the coast, resulting in low genetic differentiation among populations. Reproduction in *S. serrata* occurs by direct development. The larvae hatch as completely metamorphosed juveniles after developing in an egg case for approximately 28 days (Chambers and McQuaid, 1994b). The low dispersal potential of both the larval and adult stages of *S. serrata* is expected to result in low genetic differentiation within populations as relatives within any single population will remain near one another. Low dispersal should produce low levels of gene flow, resulting in high genetic differentiation among populations along the coast.

2.2 STUDY SITES

Figure 2.1 shows the coastline of South Africa along which sites were selected as population sampling sites for this study. Sample sites along the Cape coast begin on the west coast at Saldahna (site 1) and run south to Kommetjie (4) and then in an easterly direction past Cape Agulhas (10), the southern most tip of Africa, to Mossel Bay (15). Most of these sites are small rock platforms separated by long stretches of sandy beach. Six of these sites are located in bays: Muizenberg (5); Gansbaai (7); Struisbaai (11); Witsand (13); Stilbaai (14) and Mossel Bay (15). Sites between Saldahna (1) and Pearly Beach (8) were characterised by sub-tidal kelp beds. Sample sites along the Transkei coast begin at Hamburg (16) and extend through to Durban (32). Rocky shores dominate the Transkei coast and all the samples were taken from large wave-cut platforms. Samples from the eastern edge of the Transkei coast (30 to 32) were used only in the mtDNA sequencing analysis.

Ocean currents have been shown to play an important role in the dispersal of marine planktonic larvae along coastlines (Behrens Yamada, 1977; Bertness and Gaines, 1993; Kojima *et al.*, 1997; Brierley and Brandon, 1999; Hilbish *et al.*, 2002). As shown in Figure 2.2, the Agulhas and Benguela currents make up the major oceanic current systems around South Africa. The Agulhas Current stems from recirculation of water within an anticyclonic sub-gyre in the South West Indian Ocean, as well as from water originating from the Mozambique Channel and the Madagascar Current (Duncombe Rae, 1991). The Agulhas Current flows southwards along the southeastern coast of South Africa, following the 200m isobath. From Port Edward to Morgan's Bay (site 19 on Figure 2.1), along the Transkei coast the current is only 5 to 10 km offshore (Duncombe

Rae, 1991) as the continental shelf is narrow in this region. Flow is almost entirely south-going with high velocities of approximately 3 knots (Harris, 1978). Around Port Edward, there is some coastal discontinuity and often north-going currents are found within 2 to 3 km of the shore (Harris, 1978). South of East London, the continental shelf widens rapidly. At the widest point of the shelf, the Agulhas Current is approximately 270km offshore and its influence on the shore becomes minimal (Harris, 1978; Schumann *et al.*, 1982; 1988).

On moving westwards towards Cape Agulhas, the frequency of currents with velocities of more than one knot becomes low and between Mossel Bay and Cape Agulhas these become rare. The Agulhas Bank, formed by the widening of the continental shelf, is shallow (average depth 100m). The influence of the Agulhas Current on inshore water becomes very reduced in this region and wind stress becomes more important (Harris, 1978; Schumann *et al.*, 1988). These low current velocities provide evidence that wind has an important role in controlling the currents in this area. The presence of cold currents during southeasterly winds is well known between Port Elizabeth and Cape Agulhas. This leads to upwelling particularly between Port Elizabeth and Mossel Bay. Upwelling occurs in the lee of major capes and the upwelling water flows on shore (Beckley, 1983). Any influence by the Agulhas Current in this area is likely to be minimal (Schumann *et al.*, 1982). There are great seasonal differences in the currents along the Cape coast. Between Port Elizabeth and Cape Agulhas west-going currents are more frequent than east-going currents, though this may vary during winter (Harris, 1978). These west-going, onshore currents also appear to be more frequent around Mossel Bay than closer to Cape Agulhas (Harris, 1978).

After following the continental shelf, the Agulhas Current turns sharply near the southern most tip of Africa. The current turns eastwards in a large anticyclonic loop, known as the Agulhas retroflection (Duncombe Rae, 1991). This returns to the South Indian Ocean as the Agulhas Return Current. In the coastal area between Cape Agulhas and Cape Town, north of the Agulhas Current, wind-driven currents are important. Cells of circulation of nearshore currents form and these are generally much larger than those along the Transkei coast. The most noticeable nearshore circulation currents occur on either side of Gansbaai (population 6; Figure 2.1), and south of Cape Town (Harris, 1978).

The Benguela Current begins with northward flow off the Cape of Good Hope and moves northwesterly away from the African coast between St Helena Bay and Alexander Bay (Duncombe Rae, 1991). It is characterised by coastal upwelling and Cape Agulhas is considered to be the most appropriate boundary between the Benguela and Agulhas systems (Brown, 1992; Emanuel *et al.*, 1992; Largier *et al.*, 1992). This is partly supported by the fact that there is a rapid change of intertidal flora and fauna on either side of Cape Agulhas (Christensen, 1980). Evidence of warm water frequently "leaking" from the Agulhas system north-westerly up the west Agulhas bank, around Cape Town and up the west coast, sometimes as far as St Helena Bay, has been found (Duncombe Rae, 1991; Largier *et al.*, 1992). This coastal area between Cape Agulhas and St Helena Bay is greatly influenced by prevailing winds. In the summer months, southeasterly winds induce upwelling of cold water along the west coast. This upwelled water forms an offshore front with Agulhas current water. The position of this front is highly variable and it will occur anywhere between Cape Point and St Helena Bay (Harris, 1978).

The general ocean area south of Africa shows intense dynamic variability (Lutjeharms and Valentine, 1988). There are a number of eddies which stem from the Agulhas Current in the subtropical convergence area south of Africa, most particularly in the retroflection and fragmentation area of the Agulhas Current (Lutjeharms and Valentine, 1988). The formation of these eddies is probably related to the underlying topography (Lutjeharms and Valentine, 1988; Duncombe Rae, 1991). These eddies are shear-edge features of the retroflection of the Agulhas Current and may move in a variety of directions, including southwards towards the Sub-Tropical convergence (Lutjeharms and Valentine, 1988). In contrast to these eddies, Agulhas rings are larger and are formed by westward penetration of the retroflection, which is followed by a sudden discontinuous eastward jump and a flow of Subantarctic or South Atlantic water into the gap (Duncombe Rae, 1991). These always drift in a northwest direction (Lutjeharms and Valentine, 1988) and have an average diameter of 300km. These Agulhas rings may be one of the main mechanisms by which water is exchanged between the Indian and Atlantic Oceans (Lutjeharms and Gordon, 1987; Duncombe Rae, 1991). The Agulhas rings move in a northwesterly direction under the influence of the Benguela system across the Cape Basin until they encounter the Walvis Ridge. Here, they are delayed for a time before they then continue moving in a westerly direction (Duncombe Rae, 1991). The rings may be absorbed by the Brazil Current some 2 to 3 years after their formation (Duncombe Rae, 1991). Aside from their possible importance in transporting warm water across oceans, these Agulhas rings also have an important local effect. If the ring moves in a more northerly direction it affects the Benguela upwelling. The ring may "capture" frontal jet water from the Benguela current and so remove eggs, larvae and even juvenile fish from the area (Duncombe Rae, 1991). This can have adverse effects on the large fishing industry of the west coast. In addition, the rings may then have an influence of the

dispersal of pelagic larvae such as that of *Siphonaria capensis*. Ocean currents play an important role in larval dispersal and the link between larval dispersal of *S. capensis* and the Agulhas and Benguela currents is discussed in detail in Chapter 6.

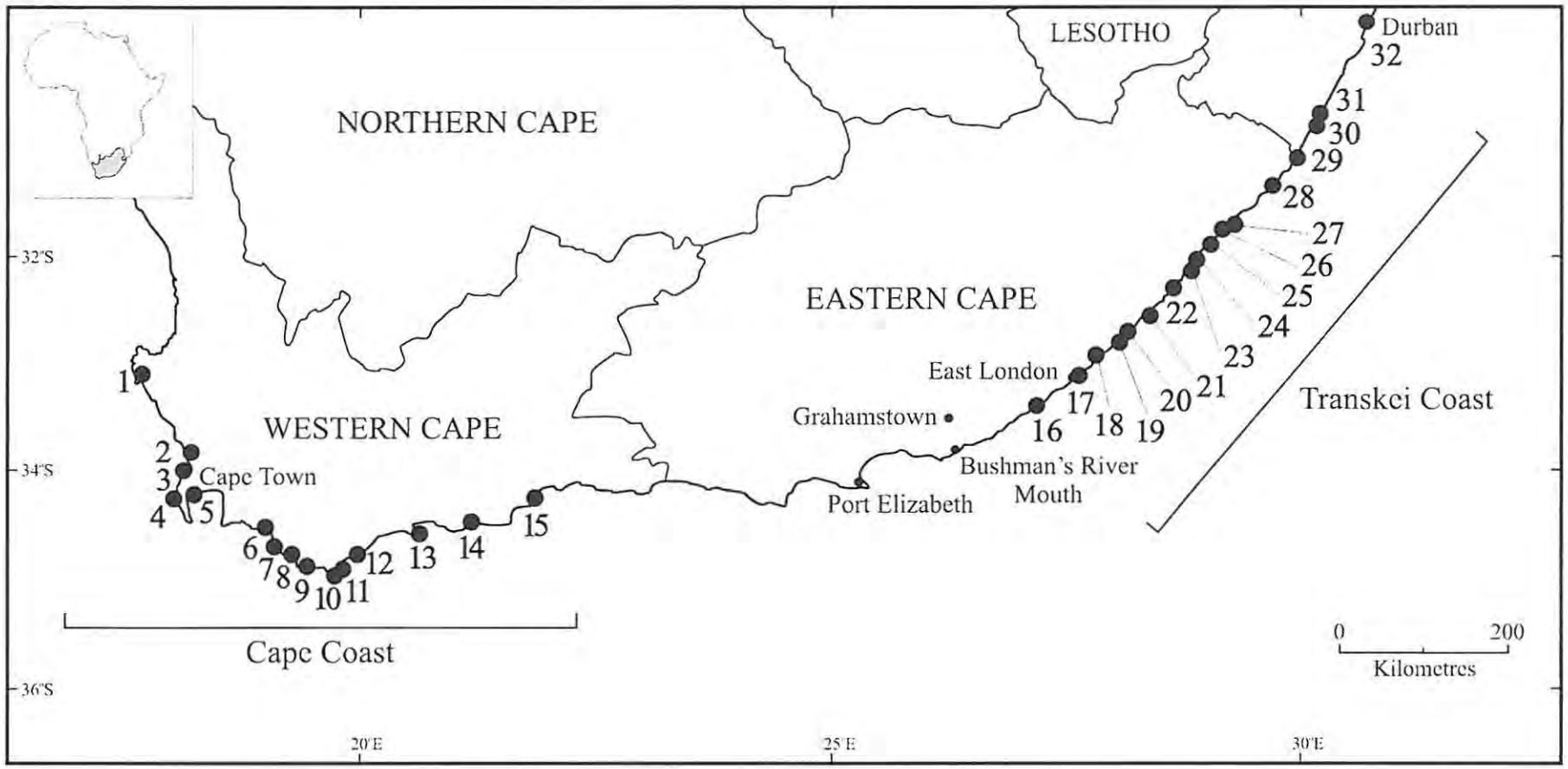


Figure 2.1: Map of the coastline of South Africa showing sample sites.

KEY: (Sites numbered from west to east)
1 Saldahna; 2 Melkbosstrand; 3 Sea Point; 4 Kommetjie; 5 Muizenberg; 6 Hermanus; 7 Gansbaai; 8 Pearly Beach; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg; 17 East London; 18 Cintsa; 19 Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 24 Coffee Bay; 25 Hluleka; 26 Umngazi; 27 Port St Johns; 28 Mkambati; 29 Port Edward; 30 Shelly Beach A; 31 Shelly Beach B; 32 Durban.

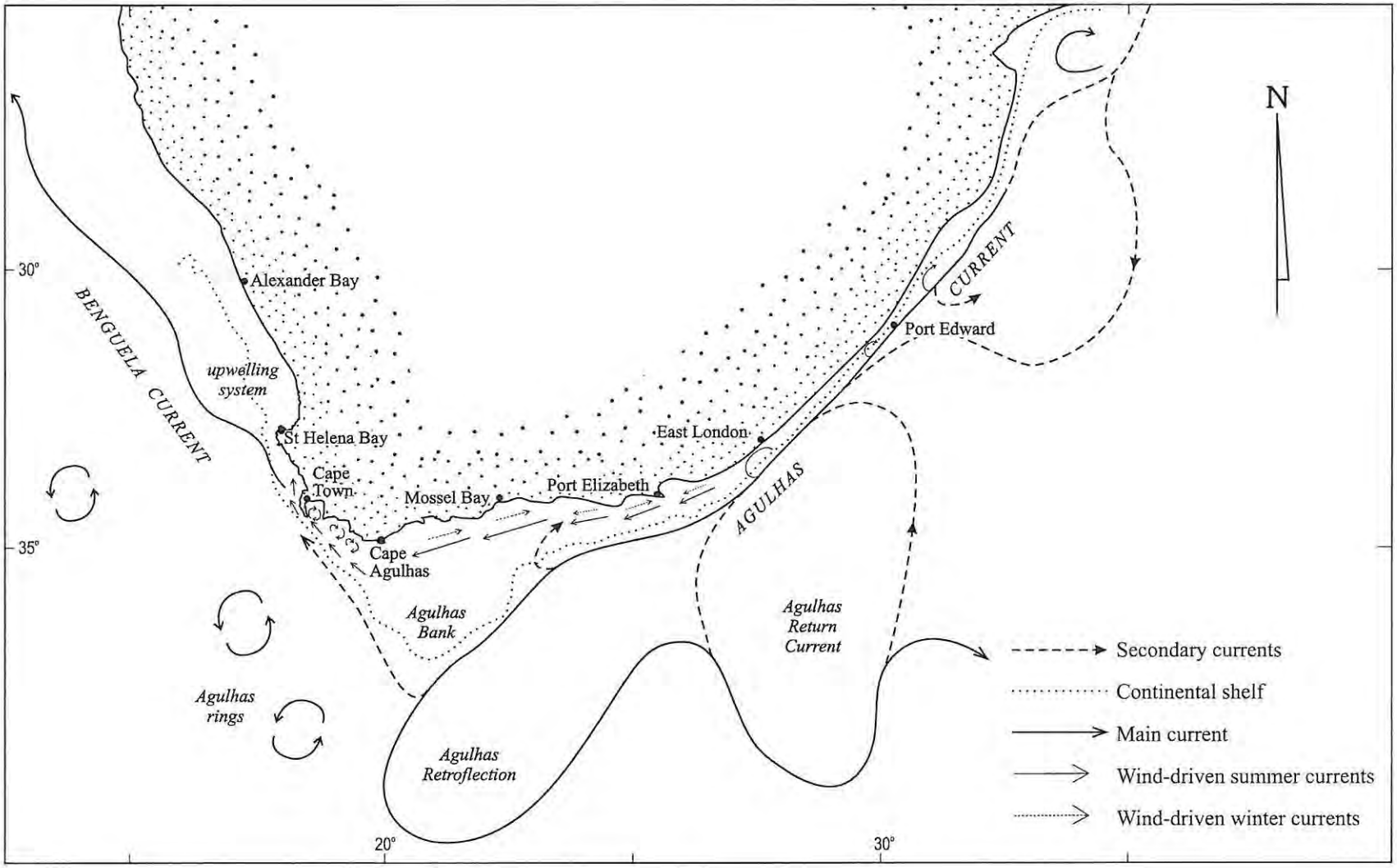


Figure 2.2: Map showing major oceanic currents around the coast of South Africa. (Modified from Harris, 1978).

CHAPTER 3: Molecular techniques -benefits & limitations

Over the past four decades, new and ever more powerful molecular techniques have led to major discoveries in the study of population biology (Arnheim *et al.*, 1990). Two significant revolutions have occurred to change the study of population biology profoundly. Firstly, in the early 1960's, protein electrophoresis was found to be useful in determining levels of genetic variation in species. This has led to an extensive literature base of allozyme studies (Amos and Hoelzel, 1992; Neigel, 1997) on virtually every type of living organism (e.g. Levinton and Suchanek, 1978; Guries and Ledig, 1982; Ward, 1990; Grant *et al.*, 1992; Klein and Seitz, 1994; Spencer *et al.*, 1997; Swart and Ferguson, 1997; Ridgway *et al.*, 1998; Todd *et al.*, 1998; Arnaud and Daguzan, 1999; Brendonck *et al.*, 2000). The second revolution occurred when DNA techniques became accessible and cost efficient, mainly through the advent of the Polymerase Chain Reaction (Mitton, 1994; Kojima *et al.*, 1997).

Allozyme electrophoresis is the most common protein based genetic technique used, although some studies have examined population differentiation through the variation in total cellular/soluble proteins within organisms (e.g. Andersen *et al.*, 1987; Nxomani *et al.*, 1994; Petit *et al.*, 1995; Plomion *et al.*, 1995; Chambers *et al.*, 1996; Brown, 1999). There is a variety of DNA techniques now available to examine variation within the genome of organisms and these include: Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), microsatellites, sequencing (of

nuclear or mitochondrial DNA) and most recently Amplified Fragment Length Polymorphisms (AFLP).

Without describing the process involved within each of these techniques, the advantages and disadvantages of each technique will be examined:

1. Total soluble proteins:

This technique is extremely simple and inexpensive (Nxomani *et al.*, 1994; Chambers *et al.*, 1996; Brown, 1999). However, there is a danger that the variation found may not be genetically based, due to post-translational modifications or environmental effects (Burton, 1983). The storage and method of collecting specimens may also cause modification of some proteins (Brown, 1999). This technique may prove more useful when used in two dimensions, where the proteins are separated both by charge and molecular weight (Petit *et al.*, 1995; Plomion *et al.*, 1995; De Vienne *et al.*, 1996).

2. Allozyme analysis

This is a very reliable technique. The genetic basis of most of the enzymes is well understood and the techniques for statistical analysis are well developed (Neigel, 1997; J W H Ferguson, *pers comm*). No formal genetic information is required to carry out allozyme electrophoresis, so this technique may be carried out on virtually any species (Neigel, 1997). An added advantage of this technique is the huge database available for comparisons of numerous loci (e.g. Nevo *et al.*, 1984). Generally, the differences within allozyme loci are expressed phenotypically and so this technique also allows investigation of the role of selection (Amos and Hoelzel, 1992). This technique is among the most cost efficient methods available (Hillis and Moritz, 1990), but often reveals very little genetic variation, and expression of some enzymes may be seasonal. Another

disadvantage is that, although there is now the technology to stain for over one hundred loci, this is a small proportion of the entire genome (Hillis and Moritz, 1990; Liu and Furnier, 1993).

Generally, the advantages of DNA techniques over protein-based analyses include the fact that variation is comparable no matter where the tissue is taken from within the organism, and analysis can be carried out on very small amounts of tissue (Hillis and Moritz, 1990; Amos and Hoelzel, 1992). The main disadvantages of any DNA analysis are expense and the length of time required in the laboratory (Amos and Hoelzel, 1992). Also, methods of analysing DNA sequence data are still under development and not as robust as those for allozyme analysis (Neigel, 1997). However, using a DNA technique allows both coding and non-coding regions of the genome to be examined, ultimately allowing more markers to be investigated (Hillis and Moritz, 1990; Liu and Furnier, 1993; Mitton, 1994). In addition, the noncoding regions often have high rates of evolutionary change (Hillis and Moritz, 1990). A useful aspect of DNA analyses is the fact that the sequence being examined may be selected on the basis of evolution rate and mode of inheritance (Hillis and Moritz, 1990).

3. RFLP

As with protein analysis, this is a relatively inexpensive and easy technique (Hillis and Moritz, 1990) but it offers little more genetic resolution, especially if used on the mtDNA genome (Hillis and Moritz, 1990; Amos and Hoelzel, 1992). A disadvantage of RFLP is that restriction enzymes are sensitive to methylation of bases in the DNA and this may cause a change in the RFLP pattern. This can be overcome by using different restriction enzymes (Hillis and Moritz, 1990). Another limitation of this technique is that it requires

fairly large quantities of DNA (Chalmers *et al.*, 1992; Hadrys *et al.*, 1992).

The Polymerase Chain Reaction (PCR) has revolutionised molecular biology and replaced some very time consuming techniques. It allows amplification of very small amounts of DNA and also of a selected sequence, while non-target sequences are not appreciably amplified. The ease, speed and sensitivity of PCR make it suitable for large population studies (Kocher *et al.*, 1989; Arnheim *et al.*, 1990; Harrison, 1991). Arnheim and co-authors in 1990 suggested that PCR was likely to disperse rapidly through the community of population biologists and indeed the last decade has proved this prediction to be true. There is an enormous amount of molecular literature utilising PCR, ranging from systematics to population biology of almost any type of organism imaginable.

4. RAPD

This technique is most useful when examining an organism for which very little, if any genetic information is available (Hadrys *et al.*, 1992; Tassanakajon *et al.*, 1997; Oakey *et al.*, 1998; Mueller and Wolfenbarger, 1999). Using the RAPD technique, relatively little time and expense are required to find patterns of variation from many (virtually an inexhaustible supply) of independent nuclear markers, from both the coding and noncoding regions of the genome (Williams *et al.*, 1990; 1993; Harrison, 1991; Clark and Lanigan, 1993; Plomion *et al.*, 1995; De Wolf *et al.*, 1998b). As with most DNA techniques, very little DNA is required (Hettle *et al.*, 1997; Oakey *et al.*, 1998). Unfortunately, RAPD is a sensitive technique and reproducibility of amplification products varies with any change in experimental conditions (Caetano-Anolles *et al.*, 1992; Hadrys *et al.*, 1992; Meunier and Grimont, 1993; Schierwater and Ender, 1993; McEwan *et al.*, 1998). However it is argued that, if handled meticulously, RAPDs are

reproducible (Meunier and Grimont, 1993; Rus-Kortekaas *et al.*, 1994; Micheli *et al.*, 1994; Brown, 1999). Unfortunately, the analytical power of this technique is low (Lynch, 1990; Hadrys *et al.*, 1992), though competitive with RFLPs. Another disadvantage of RAPD analysis is the risk of co-migration of fragments of nearly identical size but of different nucleotide sequences (Lynch and Milligan, 19; Rieseberg, 1996; Hettle *et al.*, 1997; Oakey *et al.*, 1998). This can result in an over-estimate of homology among individuals.

5. Microsatellites

These are variable numbers of tandem repeats within the nuclear genome of any organism. They are defined as micro- or minisatellites according to their size (Neigel, 1997). Microsatellites provide extremely accurate results and are useful for determining degree of variability in populations (Beyermann *et al.*, 1992; Guerin *et al.*, 1993; Spencer *et al.*, 1997) as they are relatively easy to survey in populations and are often highly polymorphic (Scribner *et al.*, 1994; Neigel, 1997). A drawback of this technique is the lengthy development time required as the correct probe needs to be developed to give high polymorphism levels (Schlotterer *et al.*, 1991; Rus-Kortekaas *et al.*, 1994). Added to this is the fact that primers/probes are seldom useful in more than a handful of closely related species (Neigel, 1997) and so must be developed for each species. Another limitation when using microsatellites is the fact that many of the genotypes found are rare and it is often not possible to test directly for Hardy-Weinberg equilibrium etc, and pooling of rare alleles for analysis can cause loss of information (Spencer *et al.*, 1997).

6. mtDNA sequencing

Advantages to using sequencing are that it examines the very basis of genotypic variation, i.e. the nucleotide bases (Hillis and Moritz, 1990), and that it provides very high resolution (Harrison, 1991). On a practical basis, since the advent of PCR, mtDNA has become easily accessible for sequencing as it is present in high enough frequencies to be used from a preparation of total cellular DNA (Hillis and Moritz, 1990; Neigel, 1997). A disadvantage of this technique is that specific primers for the gene being sequenced are needed. This can increase the developmental time considerably (Hills and Moritz, 1990). However, with an ever-increasing number of universal primers becoming available, this limitation is less problematic.

7. AFLPs

The advantage of this new technique, which combines RFLP and RAPD, is that it is possible to generate hundreds of replicable markers from the DNA of any organism and, as with RAPD, no prior knowledge of the genomic make up of the organism is needed (Mueller and Wolfenbarger, 1999). Unlike RAPD analysis, this technique shows very high reproducibility and is fairly time and cost-efficient (Kaplan *et al.*, 1998; Mueller and Wolfenbarger, 1999). The widest application of this technique is below species level and very little DNA is required for analysis.

The selection of an appropriate technique when examining a particular hypothesis is affected by the "level" at which genetic variation is being examined. In this study, genetic variation is being viewed at a population level and so the comments on choosing a suitable technique will revolve around intraspecific variation.

Allozyme analysis has been the standard technique for examining variation in populations (Spencer *et al.*, 1997), gene flow, species boundaries and breeding structures for the last 30 years (Hillis and Moritz, 1990). A decade ago, allozymes and RFLP seemed most appropriate (including the cost and efficiency) for intraspecific studies, as DNA sequencing was considered expensive and required large sample sizes (Hillis and Moritz, 1990).

RAPD analysis is particularly useful for population genetic studies (e.g. Chalmers *et al.*, 1992; Apostol *et al.*, 1993; Megnegneau *et al.*, 1993; Adamkewiez and Harasewych, 1994; Jones *et al.*, 1994; Plomion *et al.*, 1995; Stothard and Rollinson, 1996; Cunningham and Mo, 1997; Dahle *et al.*, 1997; D'Amato and Corach, 1997; Spencer *et al.*, 1997; Todd *et al.*, 1997; Huang *et al.*, 1998; De Wolf *et al.*, 1998b; Silbiger *et al.*, 1998; von Soosten *et al.*, 1998), especially when there is little or no genetic information available on the species being examined (Mueller and Wolfenbarger, 1999). RAPD has been used to some extent for taxonomic investigations (e.g. Kambhampati *et al.*, 1992; Bardakci and Skibinski, 1994; Stammers *et al.*, 1995; Chambers *et al.*, 1998; Stothard and Rollinson, 1996; Brown, 1999; Cao *et al.*, 1999; Warnke *et al.*, 2000) but this is limited.

Microsatellites are very useful for examining patterns across closely related species or within-species variation, as they do not appear to be conserved over large taxonomic distances (Spencer *et al.*, 1997). Recently, mini and microsatellites have been shown to reveal much greater levels of genetic variability than allozymes, and are considered by some superior (Hughes and Queller, 1993; Spencer *et al.*, 1997).

Sequencing can be used for almost any problem, from intraspecific variation to systematics but this does not necessarily make it the best approach. Avise *et al.* (1987) make a case that the properties of mtDNA (i.e. its maternal, non-recombining mode of inheritance, rapid evolution and extensive intraspecific polymorphisms (Harrison, 1991)) "demand" the expansion of mtDNA sequencing from phylogenetic thinking to microevolutionary level, i.e. mtDNA sequencing should be utilised in population genetics.

AFLPs may, in time, supersede other markers, including allozymes, RAPD, RFLP and microsatellites, due to their time and cost efficiency, reproducibility and resolution. One drawback is that they primarily generate dominant rather than co-dominant markers. The high reliability of AFLPs could lead to displacement of RAPD markers, and the user-friendliness of AFLP may cause replacement of RFLPs and microsatellites (Mueller and Wolfenbarger, 1999). However, AFLP analysis is unlikely to outcompete techniques, which generate co-dominant markers, such as allozymes and microsatellites, due to their largely dominant nature.

As seen from these descriptions, there are several different techniques which can be utilised for examining genetic diversity, but no single technique is universally ideal (Mueller and Wolfenbarger, 1999). Apart from considering the level of genetic resolution required, selecting a technique for any particular study is ultimately a compromise depending on:

cost (in terms of time and expense)

generation of multiple, independent markers

reproducibility of results

technical expertise available

information available about the organism's genome

(Hillis and Moritz, 1990; Mueller and Wolfenbarger, 1999).

It has been argued that it is valuable to select more than one technique when addressing a genetic question as the techniques may or may not reveal similar levels of genetic variation (Karl and Avise, 1992; Mitton, 1994; Neigel, 1997). The following examples clearly reflect this idea. Usually more pronounced genetic differentiation is found when examining populations with a DNA technique than with allozymes (Hughes and Queller, 1993; Mitton, 1994). This was found to be the case in the American oyster (Karl and Avise, 1992), the Russian wheat aphid, *Diuraphis noxia* (Puterka *et al.*, 1993), in populations of the limpet *Patella vulgata* (Hurst and Skibinski, 1995), the Mediterranean woodmouse, *Apodemus sylvaticus* (Michaux *et al.*, 1996), in *Daphnia pulex* (Crease *et al.*, 1997), the solitary bee *Megachile rotundata* (Blanchetot, 1992) and copepod sea lice *Lepeophtheirus salmonis* (Todd *et al.*, 1997).

However, in some cases similar levels of genetic variation were found with allozyme and various DNA techniques. Such a situation was found in: aspen trees (Liu and Furnier, 1993), when examining *Colletotrichum orbiculare,* a bacterium infecting cucumbers (Correll *et al.*, 1993), in the Canary Island lizard, *Gallotia galloti* (Thorpe *et al.*, 1994), in populations of Rock-wallabies, *Petrogale assimilis* (Spencer *et al.*, 1997), in the American chestnut, *Castanea dentata* (Huang *et al.*, 1998) and also in studies on the periwinkle, *Littorina striata* (De Wolf *et al.*, 1998b).

As described already, there is a considerable diversity of techniques available for various systematic problems. However, rapid development of such techniques often gives the impression that all evolutionary problems can be solved with molecular data. This is not so. Often, inaccurate results occur when inappropriate techniques are applied (Hillis and Moritz, 1990). Hence, careful consideration needs to be taken when selecting a technique for examining a particular hypothesis. When considering how dispersal potential affects genetic variation within *Siphonaria capensis* and *S. serrata* the following techniques were selected:

1. **Morphometric analysis** was carried out to examine the genetic influence on the morphology of both *S. capensis* and *S. serrata*.

2. **Total protein analysis** was selected for its ease, lack of expense and potential to reveal genetic variation in *S. capensis* and *S. serrata* as shown by Chambers *et al.* (1996).

3. When considering allozyme electrophoresis, Mitton (1994) emphasised that protein analysis should not be forgotten or ignored as, together with DNA techniques, it may reflect the predominance of different evolutionary forces. So **allozyme analysis** was chosen for its known usefulness in population studies (Hillis and Moritz, 1990; Harrison, 1991) and its relatively low cost (Hillis and Moritz, 1990). This is also a very robust technique in terms of the statistics used to analyse the data (J W H Ferguson *pers comm*).

RAPD analysis was attempted as it was considered a viable option, due to the fact that little genetic information is available for these species and, with care, reproducible results could be obtained. However, this technique proved extremely difficult as it was almost

impossible to obtain a clean total DNA extract from the limpet muscle tissue. This was attributed largely to the presence of mucopolysaccharides and defensive chemicals in the foot muscle of all *Siphonaria* species (Hodgson, 1999; McQuaid *et al.*, 1999).

4. **mtDNA sequencing** is very effective for within-species analysis, partly due to the maternal inheritance of mtDNA (Hillis and Moritz, 1990). Often this technique is not cost or time effective (Hillis and Moritz, 1990) but the use of universal primers is assisting in the increase of the efficiency of mtDNA sequencing. Hence, with some universal cytochrome *b* primer sequences available for molluscan taxa (Merritt *et al.*, 1998), this technique was successfully pursued by extracting the DNA from the ovaries of individuals.

No technique is best under all circumstances and different techniques are valuable for answering particular problems (Hillis and Moritz, 1990). Hence, morphometric analysis, total protein analysis, allozyme analysis and mtDNA sequencing were selected as complementary techniques and were found to be valuable in examining the genetic variation within *S. capensis* and *S. serrata*.

CHAPTER 4: Morphological variation

4.1 INTRODUCTION

Morphological differences between the two South African pulmonate limpet species, *Siphonaria capensis* and *S. serrata* may be caused by their different reproductive tactics. These species are closely related (Hodgson, 1999) but *S. capensis* produces pelagic larvae, while *S. serrata* is a direct developer (Chambers and McQuaid, 1994a). If the phenotype of these species is predominantly under genetic control, then it is expected that within populations of *S. capensis* phenotypic variation will be high, while among-population variation will be low due to the high level of larval dispersal, which leads to high levels of gene flow. Within populations of *S. serrata* there should be a high level of similarity in shell morphology due to the low levels of dispersal. Among-population morphological variation in this species is expected to be high with populations being easily distinguishable from one another. Such a situation was observed by Ridgway *et al.* (1999) in *Patella barbara* (planktonic developer) and in the direct developer, *Littorina saxatilis* (Snyder and Gooch, 1973; Johannesson *et al.*, 1993).

Morphological differences in the form of phenotypic variation within a species will result not only from genetic control but from a combination of genetic variation,

environmental variation, gene-environment interaction and noise (Vermeij, 1973; Newkirk and Doyle, 1975; Janson, 1982 a and b; Janson and Sundberg, 1983; Sundberg, 1988; Falconer, 1989; Palmer, 1990; Ward, 1990; Johnson and Black, 1991; Boulding *et al*, 1993; Johannesson and Johannesson, 1996; McQuaid, 1996b; De Wolf *et al.*, 1998a; Urabe, 1998; Innes and Bates, 1999). Dealing with the simplest factor first, noise includes measuring errors and possible abnormalities, which would occur as the limpet grows (e.g. partial predation and infections). This random factor is probably negligible in suitably large samples.

Varying degrees of environmental stresses such as desiccation (Newkirk and Doyle, 1975; Atkinson and Newbury, 1984; Lowell, 1984; Johannesson and Johannesson, 1996), temperature (Vermeij, 1973), wave action (Newkirk and Doyle, 1975; Atkinson and Newbury, 1984; Janson and Ward, 1984; Crothers, 1992; Grahame and Mill, 1992; Trussell *et al.*, 1993; Hobday, 1995; De Wolf *et al.*, 1998a; Innes and Bates, 1999; Tablado and Gappa, 2001) and salinity (Read, 1983; Brandwood, 1985) have been found to affect the shell morphology of intertidal marine gastropods (Branch, 1981; Sundberg, 1988). The most influential environmental factors appear to be wave action (particularly on exposed shores or high on shores) and desiccation (particularly on sheltered shores) (Newkirk and Doyle, 1975; Janson and Sundberg, 1983; Johannesson, 1986; Sundberg, 1988; Boulding *et al.*, 1993; Tatarenkov and Johannesson, 1994).

Biotic environmental factors have also been correlated with shell morphology. These include: predation (Reimchen, 1982; Vermeij, 1982b; Cook, 1983; Brandwood, 1985; Palmer, 1990; McMahon, 1992; Boulding *et al.*, 1993; Johannesson and Johannesson,

1996), primary productivity (which has a strong influence on growth rates) (Boulding *et al.*, 1993; Tablado, *et al.*, 1994), competition (Sundberg, 1988; Tablado, *et al.*, 1994; Tablado and Gappa, 2001) and population density effects (McMahon, 1992). Generally, the most important biotic factor on sheltered shores is predation (Janson, 1982a; Janson and Sundberg, 1983; Johannesson, 1986; Palmer, 1990; Boulding *et al.*, 1993; Trussell *et al.*, 1993). Crabs are often the most important predators and predation can result in selection for thicker shells (Reimchen, 1982; Vermeij, 1982b; Brandwood, 1985; Johannesson, 1986) and may also directly lead to morphological adaptations through response of the prey to predator effluents (Palmer, 1990).

As already stated, morphological differences within a species may be correlated with genetic differentiation among populations (Struhsaker, 1968; Newkirk and Doyle, 1975; Vermeij, 1982a; Janson and Ward, 1984; Grahame and Mill, 1992; Chakir *et al.*, 1995; Johannesson and Johannesson, 1996; McQuaid, 1996b) and may depend on the parentage of the individuals. Dispersal rates are known to play a fundamental role in establishing the genetic structure of a species, especially littorinid snails (Berger, 1973; Janson, 1985b; Johannesson and Johannesson, 1990) and probably most prosobranch gastropods (Macloed *et al.*, 1985; Grant and Utter, 1987; Ward, 1990; Liu *et al.*, 1991; Williams and Benzie, 1996). Wide dispersal among populations through pelagic larvae is likely to result in high gene flow and hence similar levels of genetic and morphological differentiation among widely spread populations of a species. This was found in *L. littorea* (Janson, 1987) and *Patella barbara* (Ridgway *et al.*, 1999). Limited dispersal may promote partial genetic differentiation resulting in phenotypic clines (Johannesson *et al.*, 1993) and may eventually cause reproductive isolation (Behrens Yamada, 1989), allowing populations the opportunity to adapt

genetically to peculiar environments. This will enhance genetic differentiation between populations (Snyder and Gooch, 1973; Janson, 1985b; Behrens Yamada, 1989). This genetic differentiation may be seen as enhanced shell colouring and sculpturing in local races (Grant and Utter, 1987). Such genetically controlled morphological differences were seen in *L. saxatilis*, where considerable morphological variability was found over distances of as little as to 2km (Reid, 1996).

Of course, genetic and environmental influences on phenotype are not mutually exclusive. As mentioned previously, the gene-environment interaction will also affect the phenotypic appearance of individuals. Adaptation to the local environment may result in genotypes becoming better suited to one location but not to another. The gene-environment interaction will depend largely on selection and will be affected by conditions such as pleiotropy and, more importantly, the plasticity of the genotype (Falconer, 1989). Ultimately, this gene-environment interaction will result in an increase in morphological variation between locations.

Morphometrics is a mathematical description of the form of organisms and allows for the study of relationships of body measurements, which contain correlations (James and McCulloch, 1990). In the past, ratios of various body measurements were used to illustrate variation in morphology (e.g. Newkirk and Doyle, 1975; Smith, 1981). More recently, regression analysis has been used to compare morphologies within or between species (Sundberg, 1988; James and McCulloch, 1990). However, both these methods have limitations as they do not necessarily remove the effects of size (Bookstein, 1989). Multivariate analyses have been found to be convenient alternatives, as they allow for simultaneous analysis of many variables (Sundberg,

1988; James and McCulloch, 1990; Armbruster, 1995; Baitanas *et al.*, 2000) and the effect of size is usually expressed as a single component (Bookstein, 1989; Fairbairn, 1992). Traditionally, morphometric analysis has been widely used to examine relationships among populations (Halbrendt and Brown, 1994; Kinsey *et al.*, 1994), geographic variation (Janson, 1982a; Janson and Sundberg, 1983; Janson, 1985b; Grahame and Mill, 1989; Johannesson *et al.*, 1993; Klein and Seitz, 1994; Borges, 1995; Mukaratirwa *et al.*, 1998; Johnson and Black, 1999), racial differentiation (Borges, 1995), population systematics (Borges, 1995) and separation of species (Cook, 1983; Janson, 1985b; Janson and Ward, 1985; Ward and Janson, 1985; Takada, 1992; Armbruster, 1995; Barbieri *et al.*, 1995; Langan-Cranford and Pearse, 1995; Mukaratirwa *et al.*, 1998; Ridgway *et al.*, 1998 and 2000; Villet *et al.*, 1999).

Morphological clines exist within numerous gastropod species on a variety of spatial scales. These occur on a global scale, from tropical to temperate latitudes, through to a small scale, from low to high shore levels (Vermeij, 1973). On a global scale, *Littorina scutulata* (Behrens Yamada, 1989), *L. striata* (De Wolf *et al.*, 1998a) and *L. saxatilis* (Brandwood, 1985; Janson, 1985b) all exhibit morphological differences over hundreds of kilometres, but large scale latitudinal trends are not easily distinguished in siphonariid limpets (Vermeij, 1973). However, on a small scale, a general increase in shell height and sculpturing, and a decrease in basal dimensions were seen with increasing shore height in a comparison of two species, *Siphonaria lessoni* and *S. pectinata* (Vermeij, 1973). Field observations suggest this is also true along the South African coast. *Siphonaria capensis* occurs lower on the shore and has smooth, broad shells and *S. serrata* occurs higher up shore, is generally smaller and can show elaborate shell sculpturing. Over a matter of metres between intertidal

zones, populations of the periwinkle *L. saxatilis* (Snyder and Gooch, 1973; Janson, 1982b; Janson and Sundberg, 1983; Janson and Ward, 1984 and 1985; Johannesson, 1986; Johannesson *et al.*, 1993), the Atlantic dogwhelk, *Nucella lapillus* (Palmer, 1990), and a Californian limpet, *Lottia digitalis* (Hobday, 1995) show remarkable morphological differences. A few species, including *L. saxatilis* and *L. arcana*, have been found to show morphological differences over both local and regional scales (Grahame and Mill, 1992).

Morphometric analysis was used to determine if mode of reproduction, through its effect on genetic differentiation within a species, influences variation in shell morphology of *S. capensis* and *S. serrata.* The environment and the gene-environment interaction may also contribute to the morphology of these limpets. While the two species may respond differently, it is reasonable to expect them to react to environmental conditions in a similar manner. So, if the environment plays a large role in morphological variation, it is expected that populations of both *S. capensis* and *S. serrata* will show similar levels of morphological variability along the coast in response to environmental conditions.

4.2 METHODS

Fifty individuals of each species were collected at sites located along the Cape and Transkei coasts of South Africa (Figure 2.1; Chapter 2). These individuals were collected as close to the low water mark as possible during a spring tide, within an area of approximately $1m^2$ at each site for each species. These $1m^2$ areas were located as close together as possible. This collection method minimised possible

environmental variation in limpet size, within and between species. This is important, as Hobday (1995) found that limpet size increases with tidal height and this could potentially affect the results. The individuals were immediately frozen in liquid nitrogen or preserved in 70% ethanol.

Measurements (within 0.1mm precision) were taken of length, width, height, distance from the apex to the anterior of the shell, distance from the siphon to the anterior, number of ribs and length of siphon ribs for each individual (Figure 4.1). It was found that length of the siphon ribs did not vary among individuals or populations and so this variable was excluded from the analyses. Before multivariate analyses were carried out on the data, all measurements were $\log_{10}$-transformed to linearize the effects of allometric growth (Sundberg, 1988; Bookstein, 1989; Boulding *et al.*, 1993; M H Villet, *pers comm*) and to give maximal separation of the groups of individuals (James and McCulloch, 1990).

Multivariate analyses allow the study of joint relationships between variables in data, which contain intercorrelations (James and McCulloch, 1990). Such analyses can reduce several variables to a few components, which then summarize most of the information and differentiate between groups far better than any two or three variables do alone (Boulding *et al.*, 1993). The following multivariate analyses were carried out on the data.

4.2.1 Principal Component Analysis (PCA)

This is a type of multidimensional scaling and was performed using a correlation matrix, which removes the effects of the measuring units. This analysis reduces the number of variables in the data set by finding linear combinations of the original variables that explain most of the existing variability in the form of principal components (James and McCulloch, 1990; Johnson and Wichern, 1998). When the variables are highly correlated, as is the case with shell dimensions, there will be 2 or 3 linear combinations of these variables, which explain the variation as effectively as the entire data set. This analysis was carried out to establish if any pattern of morphological variation occurred along the coast among populations of each species. Presuming a large component of genetic control of shell morphology, it can be predicted that among populations of *S. capensis* no spatial trend or pattern will be apparent, as larval dispersal will result in high gene flow. Populations of *S. serrata* are expected to show a pattern of differentiation along the coast, due to the limited dispersal potential of this species. If environmental conditions are the determining factors in shell morphology, then it can be predicted that similar trends in morphological variation will occur for both species along the coast, as sampled populations occupied similar $1m^2$ habitats.

4.2.2 Discriminant Function Analysis (DFA)

DFA calculates abstract variables which are linear combinations of the original variables that maximise among-group variation relative to within-group variation of each population (group) (Borges, 1995). This technique is used to separate sets of variables and also to allocate the original individuals into previously defined groups (Johnson and Wichern, 1998). Such a procedure allows the generation of a

classification-of-observations table. This reveals the percentage of individuals of a particular population which are correctly classified to their population of origin and those which become misclassified as part of other populations, purely on the basis of their original morphometrics. Presuming predominantly genomic control of morphology, it is predicted that *S. capensis* populations would be similar to one another and this would result in low percentages of individuals being correctly classified into their original population. On the other hand, *S. serrata* populations should be different from one another and so, based on their original morphology, individuals should be classified into their population of origin. This would result in high percentages of correctly classified individuals within each population. Once again, if environmental factors strongly influence shell morphology, similar trends will be seen in both species.

4.2.3 Canonical Correlation Analysis (CCA)

This type of analysis is a generalisation of both a correlation and a regression (James and McCulloch, 1990). It calculates the overall correlation between the linear combinations of two sets of variables (Johnson and Wichern, 1998), which in this study are geographical location (latitude and longitude) and shell morphology. A Canonical Correlation Analysis was carried out to examine geographic trends in the morphological variation of both species and to enhance the apparent geographic cline found in the PCA. A CCA also gives an indication of the magnitude of the various environmental effects. It is expected that, if genotype controls morphological variation, populations of *S. capensis* would show a weak or no cline along the coast, due to larvae dispersing along the coast. There should be no correlation between site and morphological variation within *S. serrata*, and a chaotic pattern of variation may

be evident. If environmental factors strongly influence shell morphology, morphological trends should be parallel between the two species. This analysis can also be used to gain an idea of the magnitude of the effect of the environment on shell morphology. This is accomplished by examining the squared canonical correlation value which can be interpreted as a coefficient of determination (r^2) and this explains how much one variable (geographic location) determines the other (morphology) (S Radloff, *pers comm*).

4.3 RESULTS

The PCA carried out on data pooled for both *S. capensis* and *S. serrata* produced two important principal components. The first principal component accounted for the majority of the variation (81.7%) in shell morphology and all the original shell variables contributed almost equally and significantly to this component (Table 4.1). The second principal component was responsible for another 8.2% (Table 4.1) of the total variation and emphasised changes in the number of ribs and siphon-to-anterior distance. If all the original variables show high, similar weights in the first principal component, then the first principal component can be regarded as a summary of size and the second principal component can usually be considered a size-free expression of shape, as the components are orthogonal (Bookstein, 1989; Fairbairn, 1992; Villet *et al.*, 1999). The PCA results of the complete data set do indeed show such findings for both *S. capensis* and *S. serrata*. Hence the first principal component is largely concerned with variation in the size of the limpets and the second principal component illustrates variation in shape. A 2-way ANOVA was carried out on the scores of both principal components to establish if shell morphology varied between

the species and/or with site. Both size (principal component 1) and shape (principal component 2) were found to differ significantly between the two species and also with site along the coast (Tables 4.2 and 4.3). The pattern of variation, in terms of size, was similar between the two species (Figure 4.2). A strong parallel trend between *S. capensis* and *S. serrata* was clearly evident between Saldanha (1) and The Haven (22) on the Transkei coast. Both species also showed a dramatic change in the amount of size variation between Die Dam (9) and Cape Agulhas (10) within the Cape coastal region. Evident in both species is a marked change in amount of variation in size between Stilbaai (14) and Hamburg (16). These populations mark the boundary between the two coastal regions and are some 400km apart. Variation in shape of individuals of both species did not co-vary between localities to the same extent as variation in size. Parallels between the two species can be seen along the Cape coast between Saldanha (1) and Cape Agulhas (10) and along the Transkei coast from Hluleka (25) to Port Edward (29). There was to be much less among-population variation in shape within *S. capensis* than *S. serrata* (Figure 4.3).

To highlight the differences between the two species, further analyses, as described previously, were then carried out on each species separately.

4.3.1 *Siphonaria capensis*

The first principal component of the PCA accounted for the majority of the variation in *S. capensis* (82.1%), with the second component being responsible for only a further 7.4% of the total variation (Table 4.4). All of the original variables contributed evenly to the variation among individuals in the first principal component. This can be seen by the similar weightings and significant correlations of these variables. The

number of ribs had the lowest influence, but still contributed significantly, to the first principal component (Table 4.4). The original variables showed very disparate weightings and correlations in the second principal component. The only variable not to correlate significantly with the second principal component was height. This variable also showed a particularly weak weighting value. Number of ribs showed the greatest correlation to the second principal component while the siphon-to-anterior variable made the second most important contribution to this principal component (Table 4.4). This was particularly noticeable in the Cape coast populations (results not shown). As with the PCA of the complete data set, the PCA results of *S. capensis* show the first principal component being largely concerned with variation in size of the limpets while the second component illustrated changes in shape. As length and width increased, so the number of ribs increased and the siphon moved posteriorly. The third principal component was considered unimportant in illustrating any morphological variation in *S. capensis* as it was only responsible for 4.98% of the total variation.

Results of an ANOVA performed on the first two principal components of this PCA showed that site along the coast significantly influenced both the first ($F = 54.49$; $p < 0.0001$) and second ($F = 18.98$; $p < 0.0001$) principal component scores. There appeared to be a sharp change in the size of *S. capensis* individuals between the Cape and Transkei coastal regions (i.e. between sites 15 and 16 on Figure 4.4). However, this may simply be an effect of the sampling design, as no samples were taken over approximately 400km between Mossel Bay (15) and Hamburg (16). Within each coastal region, there were substantial changes in size. Along the western area of the Cape coast, size appeared to increase gradually in an easterly direction. East of Cape

Agulhas (10) this trend became reversed. The multiple range test (Table 4.5), shows two discrete groups of populations along the Cape coast. The populations between Saldanha (1) and Arniston (12) were found to be statistically similar, with the exception of Cape Agulhas (10). The more eastern populations of Witsand (13) and Stilbaai (14) were very similar to one another and were also similar to the Cape Agulhas (10) population. The population at Mossel Bay (15) appeared to be unique (Table 4.5). Along the Transkei coast, there were two main groups of statistically similar populations, with the division occurring between Mazeppa (21) and The Haven (22) (Table 4.5). The individuals in the population located at Coffee Bay (24) showed an enormous amount of variation (Figure 4.4). This may be at least partially due to the small sample size (10 individuals) of this particular population.

The shape of *S. capensis* individuals appeared to vary less along the coastline than size (Figure 4.5), however the coefficients of variation showed no difference between them (PC1: coefficient of variation = 0.0935; PC2: coefficient of variation = 0.0897). The variation in shape within each population overlapped with that of other populations, and so no single population could be distinguished purely by shape of the limpets, except for the Saldahna (1) population (Table 4.6). As seen by the multiple range test (Table 4.6), the largest discontinuity in shape among populations occurred between Hamburg (16) and East London (17). These populations are geographically relatively close to one another ($\pm$ 60km). This suggests that there may either be a limitation in larval dispersal or some change in environmental conditions in that region.

The DFA was used primarily to generate a classification of observations table. The

first discriminant function explained 50.9% of the variation among individuals. Length and height contributed most to this discriminant function and number of ribs was the only variable not to contribute significantly (Table 4.7). The second discriminant function accounted for 29.4% of the variability among individual limpets and all the variables contributed significantly to this discriminant function (Table 4.7).

The classification-of-observations table (Table 4.8) showed a wide range of percentages of correctly classified individuals. Populations along the Cape coast showed higher percentages of correctly classified individuals than the Transkei populations and this difference was found to be significant (t-test: $t = 2.19$, $df = 23$; $p < 0.05$). Cintsa (18), The Haven (22), Hole-in-the-wall (23), Port St Johns (27) and Mkambati (28) populations had less than 10% of their individuals correctly classified. All of these populations occur along the Transkei coastal region. Only six populations showed more than 50% of their individuals as correctly classified. Of these populations, only two occurred along the Transkei coast (Qolora Mouth; 20 and Hamburg; 16). This suggests that dispersal may be lower among populations along the Cape coast than along the Transkei coast or that the environment has a stronger effect on the Cape coast. The incorrectly classified individuals of all populations appeared to be randomly placed in populations both east and west of their original population and were not restricted to their coastal region of origin. This suggests that wide dispersal of larvae may occur, or that environmental factors may cause most of the morphological variation. Saldahna (1) and Mossel Bay (15) were the only populations to show over 70% of their individuals as correctly classified (Table 4.8).

CCA illustrated roughly how the morphology of *S. capensis* was influenced by geographic location. Longitude was found to be more important than latitude in the first canonical variate (Table 4.9). While all the morphological variables contributed to the first canonical variate, length had the highest weighting and varied greatly with longitude, i.e. decreasing from west to east (Table 4.9). Height also contributed to this variate, increasing as length decreased. Figure 4.6 illustrates how length (CV1; Table 4.9) decreased from west to east as far as Hamburg (16). This trend then becomes very weak along the Transkei coast. As already stated, the squared canonical correlation value (Table 4.9) can be interpreted as a coefficient of determination (r^2) and can be used to explain how variation in geographical location determines variation in morphology (S Radloff, *pers comm*). Approximately 35% of the variation in length and height seen in *S. capensis* can be attributed to geographic location. Within the second canonical variate, siphon-to-anterior distance and width contributed most strongly to the variation which changed markedly with latitude (Table 4.9; Figure 4.7). The second canonical variable increased with latitude as far as Cape Agulhas (10), where the trend then weakens (Figure 4.7). The r^2 value of the second canonical variate showed that only about 12% of the variation mostly in width and siphon-to-anterior distance of *S. capensis* could be attributed to geographic location along the coast (Table 4.9).

4.3.2 *Siphonaria serrata*

The PCA of *S. serrata* revealed that the first principal component was responsible for 78.6% of the total variation among individuals. The second principal component was responsible for only a further 11.4% of the total variation (Table 4.10). Thus *S. serrata* showed almost as much variation in size as *S. capensis*, but more variation in

shape (Tables 4.4 and 4.10). This confirmed the initial finding in the 2-way interaction plots of the pooled PCA (Figures 4.2 and 4.3). All the original variables showed similar weightings in the first principal component and all correlated significantly with this component. The weightings of the variables within the second principal component proved to be more variable. Apex-to-anterior was the only variable not significantly correlated to the second component. Notably, the most strongly correlated variable was the number of ribs (Table 4.10). The contributions of the original variables to the first and second principal components in *S. serrata* were similar to those of *S. capensis* (Tables 4.4 and 4.10). As for *S. capensis*, the first principal component was interpreted as size, and the second component was more representative of shell shape.

The results of an ANOVA showed that both principal components varied significantly with site along the coast (PC1: $F = 61.75$; $p < 0.0001$; PC2: $F = 100.05$; $p < 0.0001$). The size of *S. serrata* individuals varied with site but did not appear to change progressively along the coastline (Figure 4.8). Instead change in shell size appeared to be less progressive and more chaotic and site dependent. Within the Cape coastal region, there were short clines among adjacent sites (e.g. sites 1 & 2, 3 to 6; 7 to 9) and then a marked change in size between Die Dam (9) and Cape Agulhas (10) (Table 4.11 and Figure 4.8). To the east of Cape Agulhas, size steadily decreased between Struisbaai (11) and Stilbaai (14). Along the Transkei coast, no clear trend was visible. As illustrated by a multiple range test, neighbouring populations were not necessarily similar to one another (Table 4.11; Figure 4.8).

Shape of *S. serrata* individuals varied along the coastline, although there was no clear

trend (Figure 4.9). Saldanha (1) was the only distinct population and there was a change in shape between Stilbaai (14) and Hamburg (16) (Table 4.12; Figure 4.9). This change occurred in the sampling gap of approximately 400km between the two coastal regions, which is in contrast to *S. capensis* where such a change in shape occurred within the Transkei coast, between Hamburg (16) and East London (17), which are only approximately 60km apart.

In the DFA, the first discriminant function accounted for 62.4% of the variation, while the second discriminant function represented 19.0% of the variation among individuals. All the original variables contributed significantly to both discriminant functions. Height and length contributed most to the first discriminant function. Length was largely responsible for the variation in the second discriminant function (Table 4.13). This analysis was used to generate a classification-of-observations table (Table 4.14). Two populations showed a high degree (>80%) of correct classification of individuals, namely Saldanha (1) and Cape Agulhas (10). All the remaining populations had less than 60% of their individuals classified correctly. The populations of *S. serrata* within the Cape and Transkei coasts were not significantly different from one another in terms of percentage of correctly classified individuals (t-test: $t = 1.76$; $df = 25$; $p = 0.09$). This suggests that the populations within both coastal regions were morphologically similar to one another. This may be due to high, unexpected dispersal, strong environmental effects, phenotypic plasticity or directional selection.

The CCA revealed more details of the significant geographic variation of shell morphology illustrated by the box and whisker plots of the PCA (Figures 4.8 and 4.9).

As stated previously, the Canonical Correlation (Table 4.15) can be used to derive a coefficient of determination (r^2) (S Radloff, *pers comm*). This indicated that 48% of the variation in length and height found in *S. serrata* can be explained by geographic position on the coast. Closer inspection of these results showed that length and height varied dramatically with longitude (Table 4.15). As with *S. capensis*, length increased and height decreased from west to east, although this trend was very weak east of Hamburg (16) (Figure 4.10). The second correlation coefficient illustrated that 20% of the variation in length, apex-to-anterior and siphon-to-anterior distances of *S. serrata* could be explained by geographic position. Within the second canonical variate, latitude was more important than longitude (Table 4.15; Figure 4.11).

4.3.3 Comparison between species

The same shell characteristics were found to make substantial contributions to the multivariate analyses conducted for both species (Tables 4.4; 4.7; 4.9; 4.10; 4.13; and 4.15) but it is important to note that the species differed, particularly in the amounts of variation.

F-ratios of the mean square values from an ANOVA of the PCA scores, in terms of both size (PC1) and shape (PC2), give a clear indication of how the species differ from one another. (Each F-ratio is estimated by mean square {among-population variation}/mean square {within-population variation}). Similar F-ratios were found between the two species when examining variation in size ($F_{S.\ capensis}$ = 128.64/2.36 = 54.5; $F_{S.\ serrata}$ = 133.92/2.17 = 61.7). This ratio suggests that there may be a slightly greater local effect on variation in size in *S. serrata* than in *S. capensis*, as the higher F-value is a result of greater among-population variation compared to within-

population variation. A direct comparison between *S. capensis* and *S. serrata* of principal components 1 further emphasised these differences between the two species. The two species showed no significant difference in the amount of variation in size of individuals (t-test for dependent or paired samples of PC1: $t = 0.913$; $p = 0.37$; Figure 4.12) and this similarity between the two species is also seen in the coefficients of variation of the PCA (*S. capensis*: cv = 0.0935; *S. serrata*: cv = 0.0925).

In terms of variation in shape the species differed considerably ($F_{S.\ capensis} = 6.16/0.32 = 19.2$; $F_{S.\ serrata} = 23.34/0.23 = 101.5$). Clearly, within-population variation is approximately 50% lower in *S. serrata*, while among-population variation is substantially greater in *S. serrata* than *S. capensis*. This suggests that, as predicted, life history appears to have a dramatic effect on variation in shape, with limited dispersal in *S. serrata* resulting in low within-population variation and high among-population variation compared to *S. capensis*. The amount of variation in shape of individuals was shown to be significantly greater (t-test for dependent or paired samples of PC2: $t = 10.872$; $p < 0.05$) in *S. serrata* than in *S. capensis*. This was initially suspected when the second principal component (illustrating variation in shape) of *S. serrata* (11.36%) was seen to be responsible for more variation than that of *S. capensis* (7.38%). This difference is clearly illustrated in a box and whisker plot (Figure 4.13). The larger amount of within-population variation in shape in *S. serrata* is further confirmed by the coefficients of variation (*S. capensis*: cv = 0.0897; *S. serrata*: cv = 0.1715).

Disappointingly, the DFA failed to reveal any differences in population structure between the two species. A Wilcoxon matched pairs test revealed no significant

difference between species in the percentage of correctly classified individuals of each site along the coast (t = 100; z = 1.4286; p = 0.15). This suggests that either similar levels of dispersal are occurring in both species, or that the environment is overriding any genetic effect on morphology.

The CCA was primarily used to examine the geographic trend of the variation in morphology of both species. It also indicated that the environment plays a greater role in determining morphology of *S. serrata* than in *S. capensis*. This is seen by the higher canonical r^2 values for *S. serrata* (Tables 4.9 and 4.15). Although inexplicable, changes in morphology of individuals of both species were found to occur at the same sites along the coast. Changes in the first canonical variate was found to occur between Hamburg (16) and East London (17) (Figures 4.6 and 4.10), while change in the second variate occurred at Cape Agulhas (10) (Figures 4.7 and 4.11). These morphological changes at the same sites in both species could be due to a strong environmental factor, such as wave crash or parasitic infestation.

4.4 DISCUSSION

On a large geographic scale, both *S. capensis* and *S. serrata* were found to vary morphologically along the coast. As expected, the same shell characteristics were responsible for the variation within each species. Length, width and apex-to-anterior contributed significantly to the first principal components, and number of ribs and siphon-to-anterior to the second components in the PCA (Tables 4.4 and 4.10). The first principal components summarised differences in size and the second principal components expressed shape differences. This was similar to *Siphonaria lessoni*

found on the Argentinean coast (Tablado and Gappa, 2001) and to *Littorina saxatilis* (Sundberg, 1988), where size rather than shape was responsible for most of the variation found among populations. Within the DFA at least one of the same variables contributed significantly to the discriminant functions of both species. For example, height correlated significantly to the first discriminant function in both *S. capensis* and *S. serrata* populations (Tables 4.7 and 4.13). The lack of discrimination among populations within both species in the classification-of-observations tables of the DFA was unexpected. A study on *Patella granularis* showed a similar result with a DFA being unable to separate populations (Ridgway *et al.*, 1998).

Geographic clines within *S. capensis* and *S. serrata*, in terms of both size and shape, were shown by the CCA and PCA. ANOVA results of the PCA scores showed that morphological variation changed with site along the coast. However, the multiple range tests (Tables 4.5; 4.6; 4.11 and 4.12) showed similarity among the majority of populations within each species. Examining variation in size and shape separately, the coefficients of variation of PC1 (*S. capensis* = 0.0935; *S. serrata* = 0.0928) showed that variation in size was similar between the two species. Although the amount of variation in size was not significantly different between the two species, that of *S. serrata* was less than in *S. capensis*. This confirms that *S. serrata* individuals within single populations are alike and show low levels of variation. The F-ratios for variation in size also show similar levels of variation between the two species (*S. capensis* = 54.5; *S. serrata* = 61.7), with that of *S. serrata* being marginally higher than *S. capensis*. This indicates slightly less within- and more among-population variation in size of *S. serrata*. In terms of variation in shape, both species supported the hypothesis that life history strategy affected shell morphology. As expected, the

levels of within-population variation were high in the pelagic developer (*S. capensis*) and low in the direct developer (*S. serrata*) (Figures 4.12 and 4.13). The F-ratios for shape were remarkably different between the two species (*S. capensis* = 19.2; *S. serrata* = 101.5). The F-ratio for *S. serrata* populations is approximately 5 times larger than that of *S. capensis*. Again this illustrates that life history influences among-population variation in both species. Limited dispersal in *S. serrata* presumably causes high levels of among-population variation.

As discussed previously, variation in the phenotype of *S. capensis* and *S. serrata* will result from a combination of variation in the genotype, the environment and the gene-environment interaction (Falconer, 1989). While it is clear that genotype has an influence on variation in shape within both *S. capensis* and *S. serrata*, the situation in terms of variation in size is much less clear. Obviously, it will be difficult to distinguish between the effects of the environment and gene-environment interaction in this study. Even though one may open Pandora's box when drawing conclusions about causes of the morphometric patterns found (James and McCulloch, 1990), it is possible to contrast the effects of the genotype versus the environment/gene-environment interactions on the phenotypic variation in size of these two species.

In addressing the issue of the causes of morphological variation, it is obvious that the environment certainly has some impact on the morphological variation of *S. capensis* and *S. serrata*. Marked changes in overall morphology of both species occurred at the same sites along the coast (Figures 4.6, 4.7; 4.10 and 4.11) and parallel trends in the ANOVA of PC1 occurred between the species suggesting a strong environmental effect on both species. Within the CCA, the geography showed a stronger influence

on the morphology of *S. serrata* (CV1: $r^2 = 0.48$; CV2: $r^2 = 0.19$) than on that of *S. capensis* (CV1: $r^2 = 0.32$; CV2: $r^2 = 0.12$) (Tables 4.9 and 4.15). While these coefficients of determination show that approximately 40% of variation in length and height was determined by geography, the remainder is probably controlled by genotypic variation or the gene-environment interaction. In terms of variation in apex-anterior and siphon-anterior, between 10 and 20% of the variation can be explained by geography. This illustrates resounding genotypic control of variation in both species, especially in controlling shape-determining measurements.

Several studies support the idea that morphological differences are at least partially inherited (Struhsaker, 1968; Newkirk and Doyle, 1975; Grahame and Mill, 1992; Chakir, 1995; Johannesson and Johannesson, 1996). Genetic influence through the dispersal of pelagic larvae was found to result in homologous shell morphology within populations of *L. littorea* (Vermeij, 1982a; Janson, 1987) and *Patella barbara* (Ridgway *et al.*, 1999). However, it must be noted that this is not always the case for example, *Littorina striata*, a planktonic developer, showed high levels of morphological variation within populations (De Wolf *et al.,* 1998b and c), as did *L. picta* (Struhsaker, 1968). The similarity of variation in size among populations of *S. capensis* along the South African coast could be due to pelagic larval dispersal (or strong environmental effects), while that of the *S. serrata* populations may be due to alternate dispersal strategies. Rafting or floating of adults or egg masses are possible mechanisms for dispersal of direct developing species (Scheltema, 1975; Janson, 1982b; O'Foighil, 1989; Bingham and Young, 1991; Martel and Chia, 1991; Tatarenkov and Johannesson, 1994; Grant and da Silva-Tatley 1997; Ruckelshaus, 1998). Drifting is likely to contribute to increased local dispersal and recruitment,

while rafting (through the use of pieces of macroalgae, etc) may increase gene flow among distant populations as it should enhance long distance dispersal (Johannesson, 1988; Martel and Chia, 1991; Worcester, 1994). Janson (1987) suggests that rafting may more readily accomplish long distance dispersal than planktonic larvae, even though it is rare. Rafting might maintain gene flow in *S. serrata* along the coast of South Africa. Ocean currents are also known to play a role in dispersal (Shepherd *et al.*, 1992; Bertness and Gaines, 1993; Kojima *et al.*, 1997; Brierley and Brandon, 1999) and along the South African coast the Agulhas and Benguela currents influence the coastal waters (see Figure 2.2, Chapter 2). If dispersal alone is the cause of similar patterns of variation of shell size of the two *Siphonaria* species, then the fast-flowing inshore Agulhas current and its countercurrents could well be responsible for high levels of dispersal in both species along the Transkei coast. Greater morphological variation among populations would be expected in the Cape coastal region, where currents are weak and variable in direction. However, there is no strong morphological variation along the Cape coast, suggesting that dispersal and its underlying genetic influence is not the primary cause of the results found. Also, alternative dispersal events, as may occur in *S. serrata*, are rare (C D Todd, *pers comm*). Such dispersal events may prevent strong genetic differentiation occurring among populations, but it is unlikely that rafting would occur at levels sufficient to result in the high morphometric (size) similarities found among *S. serrata* populations. Added to this, any alternative dispersal strategy is not likely to result in dispersal levels as high as those found in planktonic dispersing species (Hellberg, 1995) and alternative dispersal strategies may occur in species with pelagic larvae as well as those exhibiting direct development. So, it is unlikely that the high similarity in size variation among populations within both the pelagic and directly developing

species in this study are a result of similar levels of dispersal.

Environmental factors have been shown to dominate the effects of genotype on shell morphology in a number of studies, particularly in littorinids (Janson, 1982b; Janson and Sundberg, 1983; Janson, 1987; McMahon, 1992; Johannesson *et al.*, 1993; McQuaid, 1996; De Wolf *et al.*, 1998c), in the South American siphonariid limpet, *S. lessoni* (Tablado and Gappa, 2001) and in a freshwater snail, *Semisulcospira reiniana* (Urabe, 1998). It is highly likely that environmental factors play a major role in determining shell morphology of the two *Siphonaria* species in this study. Support for this suggestion lies in surprisingly similar changes in morphology of both species along the coast (e.g. 2-way interaction plots; Figures 4.2 and 4.3 and Canonical Correlation plots; Figures 4.6; 4.7; 4.10 and 4.11). The most influential environmental effects include wave erosion (Newkirk and Doyle, 1975; Smith, 1981; Grahame and Mill, 1992; Hobday, 1995), primary productivity and temperature (both of which affect growth rates) (Janson, 1982a; Tablado *et al.*, 1994; Tablado and Gappa, 2001) and predation (Janson, 1982a; Cook, 1983; Johannesson and Johannesson, 1996).

The effects of predation can be presumed to act through selection and can therefore be considered as a gene-environment interaction. The risk of predation tends to result in less feeding and slower growth rates (Reimchen, 1982; Brandwood, 1985; Palmer, 1990). It is unlikely that predation is a major factor in influencing the morphology of these *Siphonaria* species as they reside in home scars which partially protect them from predators. More importantly, they produce mucus containing polypropionate metabolites, some of which are biologically active (Hodgson, 1999) and these deter predators (McQuaid *et al.*, 1999).

Temperature, primary productivity (affecting growth rates) and wave action can be considered as solely environmental effects. Temperature may indeed affect growth rates of *S. capensis* and *S. serrata* along the South African coast, as seawater temperature is substantially cooler along the west Cape coast. This in turn would influence the morphology of the limpets, most especially their size. Warmer temperatures should result in higher growth rates so that size of these siphonarid limpets should increase with warmer temperatures from west to east along the coast. However, both species were found to decrease in size from west to east. Primary productivity decreases along the South African coast from west to east (Bustamente *et al.*, 1995) and this, rather than temperature, may influence the growth rate of *S. capensis* and *S. serrata* (although not necessarily in the same manner). Both species decreased in size from west to east and this may well be due to decreasing food abundance. Growth rates were seen to be determined by food availability in *S. lessoni*, where sewage outfall enhanced primary production and caused significant changes in shell size (Tablado *et al.*, 1994; Tablado and Gappa, 2001).

Wave exposure often influences both size and shape of shells (Lowell, 1984; Sundberg, 1988; Hobday, 1995; Innes and Bates, 1999), high exposure resulting in larger apertures and a bigger foot for gripping onto rocks (Johannesson, 1986). It may limit size on exposed shores (Struhsaker, 1968). South African shores are known to be exposed in terms of wave action and this probably has an effect on the shell morphology of *S. capensis* and *S. serrata*. Wave exposure has not been measured directly, but it may account for the variation in morphology along the coast seen in the 2-way interaction plots (Figures 4.2 and 4.3). Also, wave action often increases

the productivity of a coastal area and this could account for the some of the morphological variation seen in these *Siphonaria* species.

It appears that the environment or the gene-environment interaction is largely responsible for the phenotypic variation seen in terms of variation in size, within both *S. capensis* and *S. serrata*. To complement this idea is the fact that shell form in marine invertebrates is known to be highly plastic (McMahon, 1992; Boulding *et al.*, 1993). If this is the case, then one genotype may produce several phenotypes, each of which will be subject to varying selection pressures in different environments. This implies that populations living in changing conditions can theoretically survive for long periods, with little genotypic change. So, while phenotypic variation can be clearly seen within both *Siphonaria* species, the genetic base within each species may be the same in all populations and variation may be due to interactions of genotype expression with local conditions.

Such a situation was observed in a study on freshwater snails of the *Bulinus truncatus/tropicus* complex (Mukaratirwa *et al.*, 1998) and in a planktonic developing periwinkle, *Littorina striata* (De Wolf *et al.*, 1998c). Both studies showed significant morphological variation among populations, but allozyme analysis revealed little genetic differentiation and supported the existence of a single species, respectively. Alternatively, large levels of morphological similarity may be found among genetically distinct groups as in the East Atlantic neogastropod *Columbella* (Oliverio, 1995) and in *Patella granularis* along the South African coast (Ridgway *et al.*, 1998). With these contrasting situations in mind, researchers often use a variety of techniques such as breeding experiments, analysis of reproductive structures and

behaviour, allozyme analysis, mtDNA sequencing and RFLPs (Johannesson and Johannesson, 1990; Boulding *et al.*, 1993; Kinsey *et al.*, 1994; Langan-Cranford and Pearse, 1995; Stothard *et al.*, 1997; Ridgway *et al.*, 1998) to examine variation within or between species. So, total protein analysis (Chapter 5), allozyme analysis (Chapter 6) and mtDNA sequencing (Chapter 7) were carried out on *S. capensis* and *S. serrata*, not only to examine the influence of developmental strategy on genetic variation, but also to confirm the strong environmental (non-genetic) effect on variation in shell size.

4.5 CONCLUSION

Clearly, within both *Siphonaria* species variation in size was more strongly influenced by environmental factors and variation in shape was more strongly influenced by genotype. Both within- and among-population variation, in terms of variation in size was found to be similar in both species. Such a result would only be expected if environmental factors influenced shell morphology and can be clearly seen by r^2 values within the Canonical Correlation Analysis. The amount of variation in shape of individuals was found to be relatively high within populations of *S. capensis* and lower within populations of *S. serrata*. Among-population variation, in terms of shape, was found to be greater in *S. serrata* than in *S. capensis*. This supported the predictions made based on the genotype having a major effect on morphological variation.

Table 4.1: Pearson's correlation coefficients (r) relating the first two components (PC1 and PC2) of a principle component analysis of the complete data of *S. capensis* and *S. serrata* to the original morphometric measurements (* indicates a significant p value).

Variable	PC1	r_1	$p(r_1=0)$	PC2	r_2	$p(r_2=0)$
Log_{10} (length)	0.1947	0.9542	<0.0001*	-0.3965	-0.1953	<0.0001*
Log_{10} (width)	0.1932	0.9466	<0.0001*	-0.3587	-0.1767	<0.0001*
Log_{10} (height)	0.1777	0.8712	<0.0001*	0.1960	0.9658	<0.0001*
Log_{10} (apex-to-anterior)	0.1939	0.9507	<0.0001*	0.0935	0.0461	<0.0001*
Log_{10} (siphon-anterior)	0.1858	0.9107	<0.0001*	-0.5166	-0.2545	<0.0001*
Log_{10} (number of ribs)	0.1584	0.7766	<0.0001*	1.1957	0.5891	<0.0001*
Eigenvalue	4.90			0.49		
% variance	81.70			8.21		

Table 4.2: Results of a 2-way ANOVA to determine if the morphology (in terms of principal component 1) of *S. capensis* and *S. serrata* differed with site along the coast and due to the species (* indicates a significant p value).

Source of variation	df effect	MS effect	F	p
Site	23	35.862	90.495	0.001*
Species	1	324.762	819.514	0.001*
Interaction between site and species	23	8.245	20.805	0.001*
Error	2282	0.396		

Table 4.3: Results of a 2-way ANOVA to determine if the morphology (in terms of principal component 2) of *S. capensis* and *S. serrata* differed with site along the coast and due to the species (* indicates a significant p value).

Source of variation	df effect	MS effect	F	p
Site	23	33.297	90.259	0.001*
Species	1	519.369	1407.853	0.001*
Interaction between site and species	23	6.028	16.339	0.001*
Error	2282	0.369		

Table 4.4: Pearson's correlation coefficients (r) relating the first two components (PC1 and PC2) of a principle component analysis of *S. capensis* populations to the original morphometric measurements (* indicates a significant p value).

Variable	PC1	r_1	$p(r_1=0)$	PC2	r_2	$p(r_2=0)$
Log_{10} (length)	0.4332	0.9617	<0.0001*	-0.1827	-0.1216	<0.0001*
Log_{10} (width)	0.4295	0.9534	<0.0001*	-0.1641	-0.1093	<0.0005*
Log_{10} (height)	0.4004	0.8887	<0.0001*	0.0153	0.0102	0.7271
Log_{10} (apex-to-anterior)	0.4183	0.9285	<0.0001*	-0.2059	-0.1371	<0.0001*
Log_{10} (siphon-anterior)	0.4086	0.9069	<0.0001*	0.2341	-0.1558	<0.0001*
Log_{10} (number of ribs)	0.3544	0.7867	<0.0001*	0.9178	0.611	<0.0001*
Eigenvalue	4.927			0.443		
% variance	82.12			7.38		

Table 4.5: *S. capensis* results of a Tukey's HSD comparison test to identify significant differences in the first principal component among localities along the coast. Localities are arranged in geographical order from west to east (X in different columns indicates no significant differences among populations).

Coastal Region	Locality	Popn No	Tukey HSD Mean	Homogenous groups
Cape	Saldanha	1	0.284	X X
	Melkbosstrand	2	0.293	X X
	Sea Point	3	0.377	X X X
	Muizenberg	5	0.715	X X X X X
	Die Dam	9	0.847	X X X X
	Cape Agulhas	10	-0.386	X X X
	Struisbaai	11	0.383	X X X X
	Arniston	12	0.240	X X
	Witsand	13	-0.653	X X X X
	Stilbaai	14	-0.356	X X X
	Mossel Bay	15	-1.551	X
Transkei	Hamburg	16	1.191	X
	East London	17	0.561	X X X X
	Cinsta	18	0.233	X X
	Morgan's Bay	19	0.621	X X X X
	Qolora	20	0.886	X X X
	Mazeppa	21	0.916	X X
	The Haven	22	-0.434	X X X X
	Hole in the wall	23	-0.012	X X
	Coffee Bay	24	-1.154	X X
	Hluleka	25	-1.004	X X
	Umngazi	26	-0.582	X X X X
	Port St Johns	27	-0.723	X X X X
	Mkambati	28	-0.918	X X X
	Port Edward	29	-1.009	X X

Table 4.6: *S. capensis* results of a Tukey's HSD comparison test to identify significant differences in the second principal component among localities along the coast. Localities are arranged in geographical order from west to east (X in different columns indicates no significant differences among populations).

Coastal Region	Locality	Popn No	Tukey HSD Mean	Homogenous groups
Cape	Saldanha	1	1.474	X
	Melkbosstrand	2	0.724	X X X
	Sea Point	3	-0.006	X X X X X X X X
	Muizenberg	5	0.578	X X X X X X
	Die Dam	9	0.050	X X X X X X X X X
	Cape Agulhas	10	0.098	X X X X X X X X X
	Struisbaai	11	-0.189	X X X X X X
	Arniston	12	0.637	X X X X X X X X
	Witsand	13	0.327	X X X X X X X X
	Stilbaai	14	0.446	X X X X X X
	Mossel Bay	15	0.142	X X X X X X X X X
Transkei	Hamburg	16	0.379	X X X X X X X
	East London	17	-0.601	X X X
	Cinsta	18	-0.485	X X X X X
	Morgan's Bay	19	-0.379	X X X X X
	Qolora	20	-0.834	X
	Mazeppa	21	-0.489	X X X X X
	The Haven	22	-0.367	X X X X X X X
	Hole in the wall	23	-0.294	X X X X X X
	Coffee Bay	24	-0.031	X X X X X X X X X
	Hluleka	25	-0.568	X X X X
	Umnazi	26	0.037	X X X X X X X X X X
	Port St Johns	27	0.619	X X X X X
	Mkambati	28	-0.291	X X X X X X
	Port Edward	29	-0.761	X X

Table 4.7: Pearson's correlation coefficients (r) relating the first two discriminant functions (DF1 and DF2) of a discriminant function analysis of *S. capensis* populations to the original morphometric measurements (* indicates a significant p value).

Variable	DF1	r_1	$p(r_1=0)$	DF2	r_2	$p(r_2=0)$
Log_{10} (length)	1.7152	0.4121	<0.0001*	-0.1745	0.8895	<0.0001*
Log_{10} (width)	0.2245	0.2513	<0.0001*	0.3625	0.9093	<0.0001*
Log_{10} (height)	-1.7007	-0.2064	<0.0001*	0.4378	0.9438	<0.0001*
Log_{10} (apex-to-anterior)	0.4682	0.2191	<0.0001*	0.2194	0.9091	<0.0001*
Log_{10} (siphon-anterior)	-0.3335	0.2448	<0.0001*	0.0094	0.8191	<0.0001*
Log_{10} (number of ribs)	-0.4254	-0.0486	0.0944	0.2998	0.8257	<0.0001*
Eigenvalue	1.967			1.135		
% variance	50.86			29.35		

Table 4.8: Classification-of-observations table of percentages of *S. capensis* populations along the entire coast line. Populations arranged from west to east along the coast. Bold numbers indicate individuals correctly classified as part of their original population, percentage of individuals classified into population other than their original population appear in rows, west or east of their origin.

	Cape coast											**Transkei coast**												
	1	**2**	**3**	**5**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
1	**72**	14		14																					
2	22	**36**	8	12		8	4	4	2								2						2		
3	6	20	**34**	12		2	14	6									6								
5	20	12	12	**35**			2										16					2			
9		2			**36**				2			20	10	8	6	12			2						2
10	2	6	4	2	2	**18**		4	6	6	6	4		12			4	4	2		4	4	4	6	
11	6	4	8	4		6	**34**	8		4		2				2	12	4	2			2		2	
12		2	8	2		4	6	**36**	6	14		10	2	2					6					2	
13	4					2	4		**56**	8								6	4		4		2	2	8
14		4				8	2	6	4	**52**			4		2			4	2		2		4		6
15	2								4	4	**76**											8	6		
16					14		2	6	4			**56**	8			4	6								
17					4			4	2	8		18	**38**	10	2	6			8						
18		2			10	2	4	2	2	6		4	4	**28**	2	14		2	6						12
19			2		6				6	2		20	10	12	**8**	18		2	8		2				4
20				2	8		2					2	12	6	2	**60**	6								
21	2	4	6	10			8	4				4	4	2	2		**49**							2	
22			4		9	9			2	4		2	4	7	2	4	2	**2**	4		13	4	20		4
23		2			4	2	2	6	12	6		6	14	6	6	6	4	8	**4**					6	6
24																	14			**14**	14		14	14	14
25						4			6	2			2	10	2	2	2	6	2	4	**22**		2	10	24
26	6		11	3													6					**34**	9	8	2
27		6	2	10		4	10	2	2		20	4				2	2				2		**6**	6	
28			2		2	4		4	13	6	2	4	2	10		2	2	2	2	2	10	2	8	**8**	8
29					2				6	4			2	2	2	4		2	2		18		8	8	**48**

Table 4.9: Results of the canonical correlation analysis of morphology of *S. capensis* populations

Coefficients	CV1	CV2
Eigenvalue	0.3508	0.1223
Canonical Correlation (r)	0.5923	0.3497
Coeff. of determination (r^2)	0.3508	0.1223
Lambda Prime	0.5698	0.8777
Chi-Square	661.74	153.48
df	12	5
p	<0.0001	<0.0001
Morphological variables		
Log_{10} (length)	2.1159	0.6696
Log_{10} (width)	-0.8767	-0.8857
Log_{10} (height)	-1.1335	-0.0179
Log_{10} (apex-anterior)	0.4786	-0.6931
Log_{10} (siphon-anterior)	-0.1323	1.2982
Log_{10} (no. of ribs)	-0.6774	0.4263
Geographic variables		
Log_{10} (latitude)	-1.1606	-1.5329
Log_{10} (longitude)	1.7885	0.7056

Table 4.10: Pearson's correlation coefficients (r) relating the first two components (PC1 and PC2) of a principal component analysis of *S. serrata* populations to the original morphometric measurements (* indicates a significant p value).

Variable	PC1	r_1	$p(r_1=0)$	PC2	r_2	$p(r_2=0)$
Log_{10} (length)	0.4292	0.9322	<0.0001*	-0.3578	-0.2954	<0.0001*
Log_{10} (width)	0.4269	0.9274	<0.0001*	-0.3063	-0.2530	<0.0001*
Log_{10} (height)	0.3928	0.8533	<0.0001*	0.3917	0.3234	<0.0001*
Log_{10} (apex-to-anterior)	0.4386	0.9528	<0.0001*	0.0525	0.0433	0.1121
Log_{10} (siphon-anterior)	0.4175	0.9070	<0.0001*	-0.3212	-0.2652	<0.0001*
Log_{10} (number of ribs)	0.3354	0.7286	<0.0001*	0.7202	0.5946	<0.0001*
Eigenvalue	4.718			0.682		
% variance	78.64			11.36		

Table 4.11: *S. serrata* results of a Tukey's HSD comparison test to identify significant differences in the first principal component among localities along the coast. Localities are arranged in geographical order from west to east (X in different columns indicates no significant differences among populations).

Coastal Region	Locality	Popn No	Tukey HSD Mean	Homogenous groups
Cape	Saldanha	1	0.011	X X X X X X
	Melkbosstrand	2	0.291	X X X X X
	Sea Point	3	-0.257	X X X X X
	Muizenberg	5	-0.127	X X X X X
	Hermanus	6	-0.225	X X X X X
	Gansbaai	7	0.338	X X X X X
	Pearly Beach	8	0.555	X X X X
	Die Dam	9	0.801	X X X
	Cape Agulhas	10	-1.600	X X
	Struisbaai	11	-0.278	X X X X X
	Arniston	12	-0.163	X X X X X X
	Witsand	13	-0.822	X X X X
	Stilbaai	14	-1.418	X X
Transkei	Hamburg	16	0.984	X X
	East London	17	0.353	X X X X X
	Cinsta	18	-0.246	X X X X X
	Morgan's Bay	19	0.689	X X
	Qolora	20	1.237	X X
	Mazeppa	21	1.654	X X
	The Haven	22	0.320	X X X X X X
	Hole in the wall	23	-0.807	X X X
	Coffee Bay	24	0.499	X X X X
	Hluleka	25	-0.098	X X X X X
	Umgazi	26	-0.641	X X X X
	Port St Johns	27	-0.790	X X X
	Mkambati	28	0.124	X X X X X
	Port Edward	29	-0.374	X X X X X X

Table 4.12: *S. serrata* results of a Tukey's HSD comparison test to identify significant differences in the second principal component among localities along the coast. Localities are arranged in geographical order from west to east (X in different columns indicates no significant differences among populations).

Coastal Region	Locality	Popn No	Tukey HSD Mean	Homogenous groups
Cape	Saldanha	1	2.041	X
	Melkbosstrand	2	0.790	X X X
	Sea Point	3	0.371	X X X
	Muizenberg	5	0.897	X X
	Hermanus	6	0.737	X
	Gansbaai	7	0.644	X X X
	Pearly Beach	8	-0.309	X X X X X
	Die Dam	9	0.102	X X X X
	Cape Agulhas	10	0.309	X X X X
	Struisbaai	11	-0.055	X X X X X
	Arniston	12	0.380	X X X X
	Witsand	13	-0.393	X X X X
	Stilbaai	14	-1.156	X X X
Transkei	Hamburg	16	0.144	X X X X
	East London	17	-0.710	X X X X X
	Cinsta	18	-1.072	X X
	Morgan's Bay	19	-1.121	X X X
	Qolora	20	-0.553	X X X X
	Mazeppa	21	-0.634	X X X
	The Haven	22	-0.575	X X X
	Hole in the wall	23	0.307	X X X X
	Coffee Bay	24	-0.680	X X X X X
	Hluleka	25	-0.340	X X X X
	Umngazi	26	0.484	X X X X
	Port St Johns	27	0.424	X X X
	Mkambati	28	0.036	X X X X
	Port Edward	29	-0.294	X X X

Table 4.13: Pearson's correlation coefficients (r) relating the first two components (DF1 and DF2) of a discriminant function analysis of *S. serrata* populations to the original morphometric measurements (* indicates a significant p value).

Variable	DF1	r_1	$p(r_1=0)$	DF2	r_2	$p(r_2=0)$
Log_{10} (length)	-0.7789	0.1779	<0.0001*	1.0309	0.7852	<0.0001*
Log_{10} (width)	-0.4111	0.2385	<0.0001*	-0.0504	0.6434	<0.0001*
Log_{10} (height)	1.3269	0.7846	<0.0001*	0.5998	0.5754	<0.0001*
Log_{10} (apex-to-anterior)	-0.0655	0.5368	<0.0001*	-0.0682	0.6276	<0.0001*
Log_{10} (siphon-anterior)	-0.1487	0.1992	<0.0001*	-0.0891	0.6623	<0.0001*
Log_{10} (number of ribs)	0.6213	0.7314	<0.0001*	-0.8568	0.0679	0.0848
Eigenvalue	3.288			1.005		
% variance	62.36			19.06		

Table 4.14: Classification-of-observations table of percentages of *S. serrata* populations along the entire coast line. Populations arranged from west to east along the coast. Bold numbers indicate individuals correctly classified as part of their original population, percentage of individuals classified into population other than their original population appear in rows, west or east of their origin.

	Cape coast													**Transkei coast**													
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**	**17**	**18**	**19**	**20**	**21**	**21**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
1	**80**	6		8	2	2																		2			
2	2	**54**	4	4	17		8	6		2														2			
3	2	6	**22**	4	4	10	6	6	8	16	4									2	4			4		2	
5	2	14	4	**46**	8		2	6		2	4										4		2	4	2		
6	12	8	8	12	**28**	12	4	2	8											2				4			
7			8	10	6	**31**	10	6	4	2	2			8				2	2		4			2		2	
8			2	4	4	4	**28**	22						20					6	6				2		2	
9		4		2	2	14	12	**52**		2	4			4					2	2							
10	2		2	2					**84**	2			2											4	2		
11		6	4	2	4	4	4	8	10	**24**	2	2	4	6	4	4				2	4				4		
12			2	8		2	2	2	4	10	**32**	8			6									2	6	10	4
13				2								**54**	4		4	10	4						4		2	6	10
14									4				**58**		2	10	4			2	4				4		8
16					2	6	12	8						**42**		2		4	10	4		2	2	2			2
17							2							2	**24**	20	10	10	2			6	12			2	6
18										4	2		4		10	**54**	2			8			2		2		6
19														2	16	4	**48**	16	4			2	6				2
20										2				10	10	2	6	**42**	14	2		4	6			2	
21							6	2						14				10	**58**								
22				2	2	6	4	8		6				8	6	8	6	2	4	**20**		6	2	2		6	2
23			8	2	2	6	4		6	6			4		2						**12**	2		18	12	6	10
24							2			2				6	8		12	10	2	10	2	**22**	4	4	4	4	8
25											2		2	2	6	2	10						**28**			14	34
26			2		6	6	4	2	6		2	2	2	4							14			**24**	12	6	2
27		2		2	2	2	2		6	2	6	8		2							12			14	**26**	10	4
28						2		2	4		4		2	12			2	4		10	2	4	12	6	4	**26**	4
29			2									2	10		4	4	2	2				4	18		4	2	**46**

Table 4.15: Results of the canonical correlation analysis of morphology of *S. serrata*.

Coefficients	CV1	CV2
Eigenvalue	0.4752	0.1932
Canonical Correlation (r)	0.6894	0.4395
Coeff. of determination (r^2)	0.4753	0.1932
Lambda Prime	0.4234	0.8068
Chi-Square	1152.14	287.76
df	12	5
p	<0.0001	<0.0001
Morphological variables		
Log_{10} (length)	-1.6300	-0.4695
Log_{10} (width)	-0.0269	-0.3528
Log_{10} (height)	0.8018	0.7236
Log_{10} (apex-anterior)	0.1949	-0.9260
Log_{10} (siphon-anterior)	0.3416	1.7664
Log_{10} (no. of ribs)	0.2170	-0.8634
Geographic variables		
Log_{10} (latitude)	0.9164	-1.8929
Log_{10} (longitude)	-1.7062	1.2295

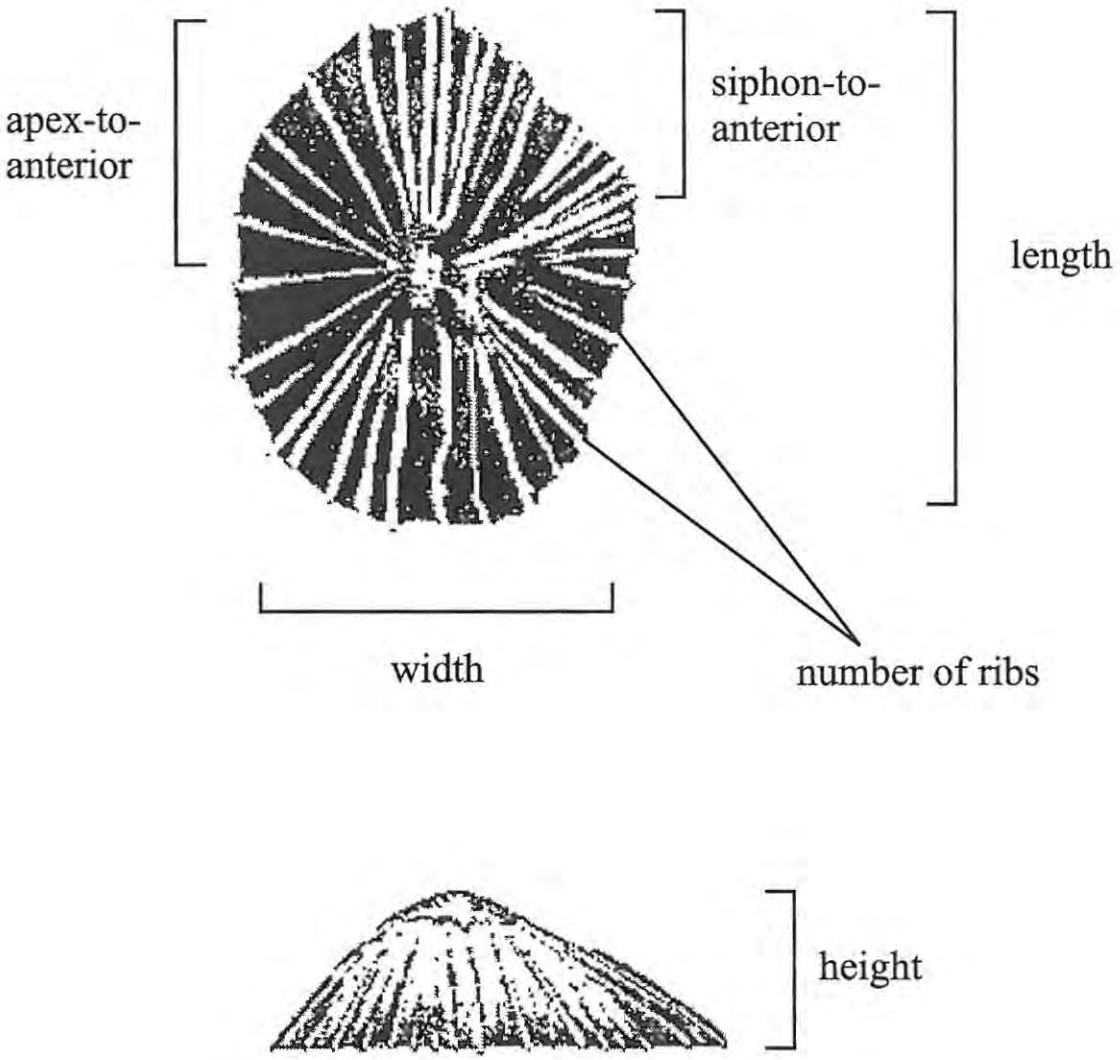


Figure 4.1: Diagram showing original measurements taken from *S. capensis* and *S. serrata* individuals for morphometric analysis.

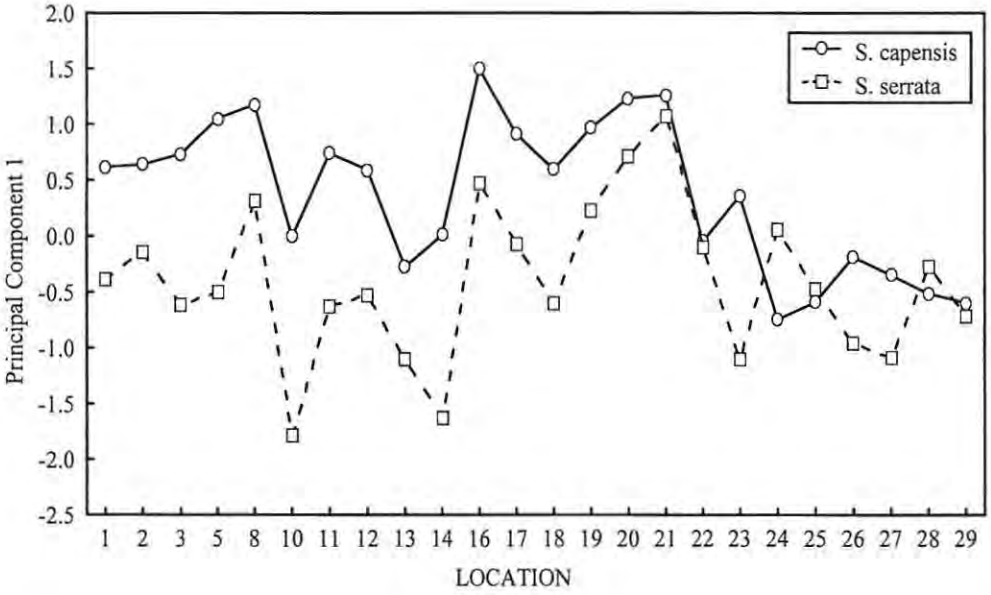


Figure 4.2: 2-way interaction plot for the first principal component of both *S. capensis* and *S. serrata*.

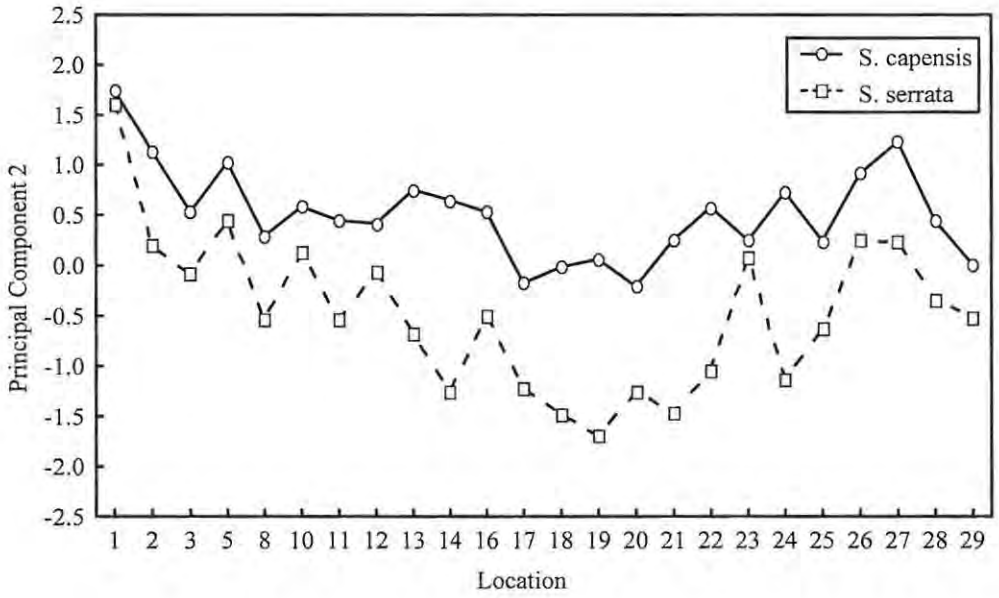


Figure 4.3: 2-way interaction plot for the second principal component of both *S. capensis* and *S. serrata*.

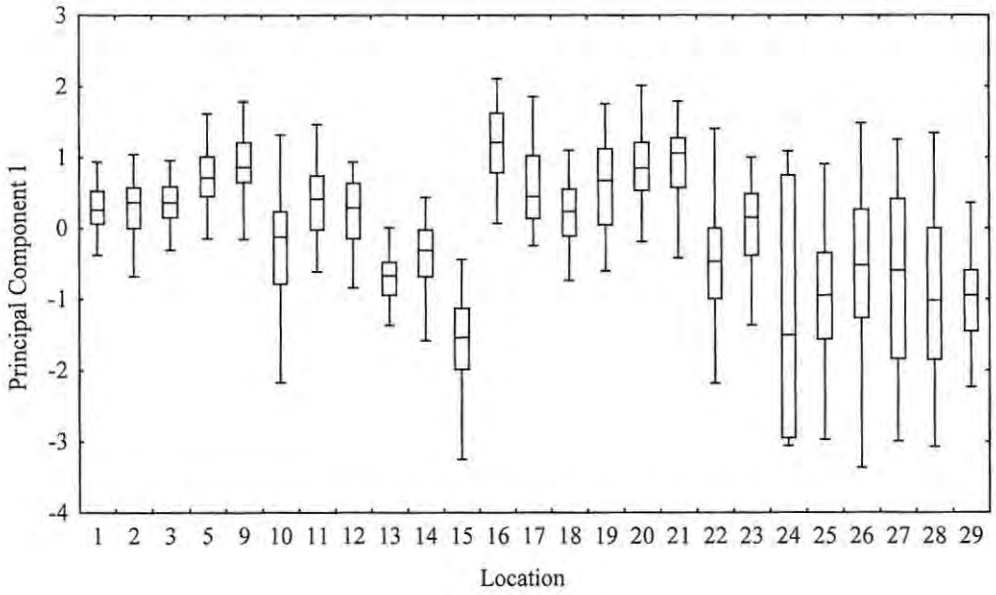


Figure 4.4: Box and whisker plot (median and IQR and range) of the first principal component score of a PCA of the morphology of *S. capensis*.

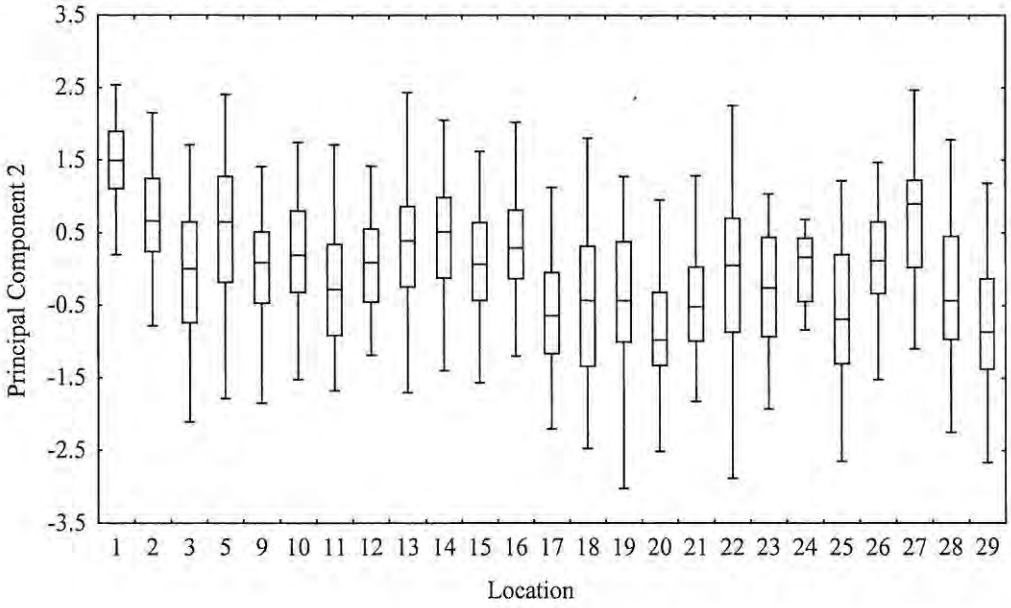


Figure 4.5: Box and whisker plot (median and IQR and range) of the second principal component score of a PCA of the morphology of *S. capensis*.

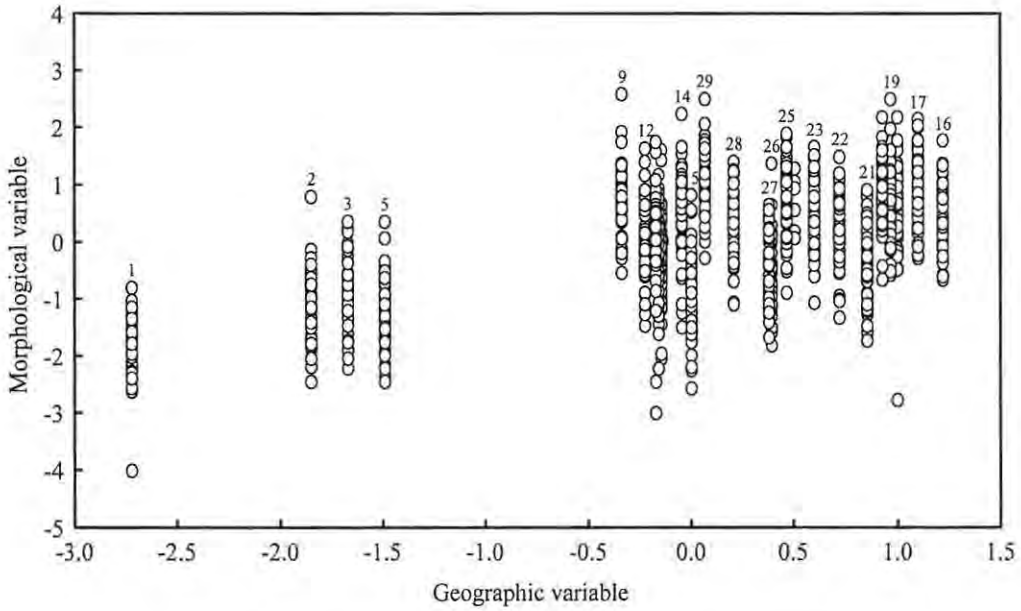


Figure 4.6: Canonical correlation plot for the first canonical variate of *S. capensis*

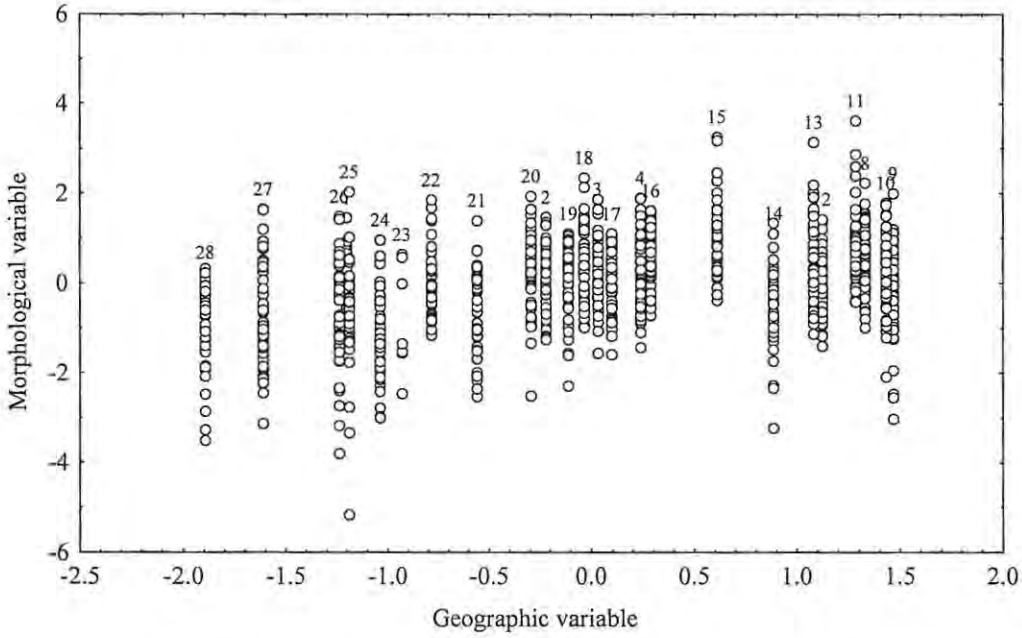


Figure 4.7: Canonical correlation plot for the second canonical variate of *S. capensis*

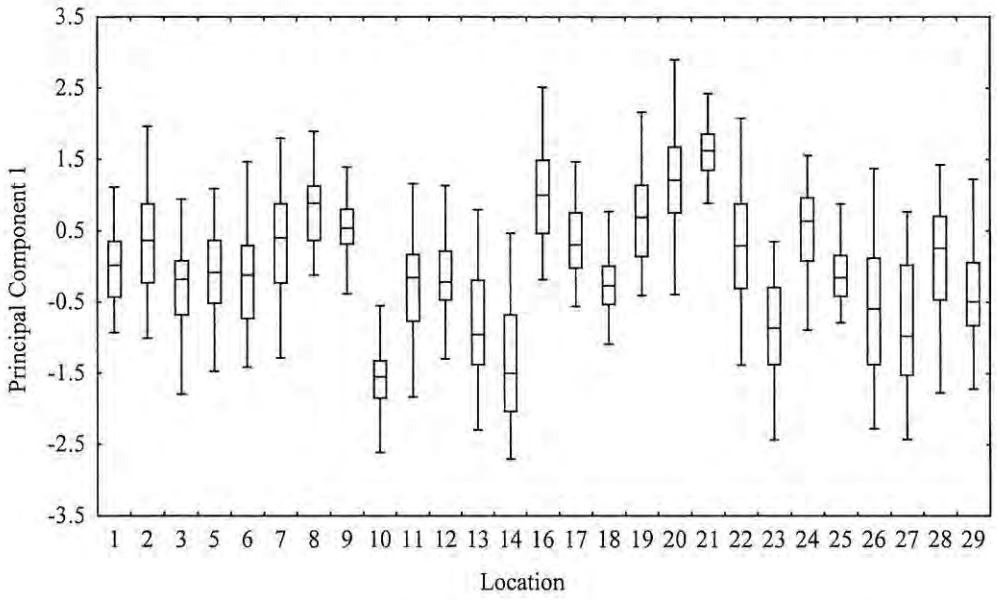


Figure 4.8: Box and whisker plot (median and IQR and range) of the first principal component score of a PCA of morphology of *S. serrata.*

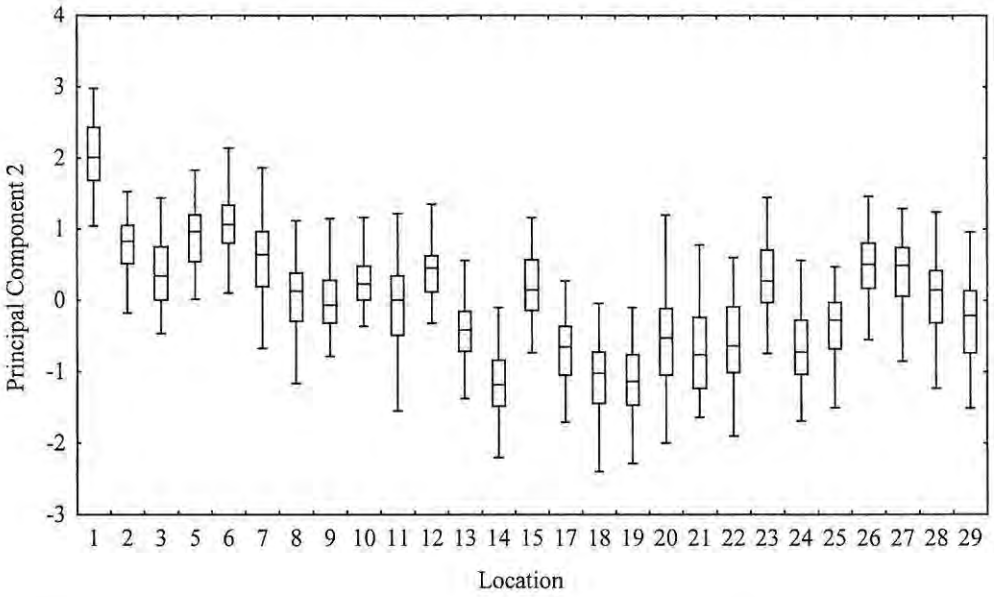


Figure 4.9: Box and whisker plot (median and IQR and range) of the second principal component score of a PCA of morphology of *S. serrata.*

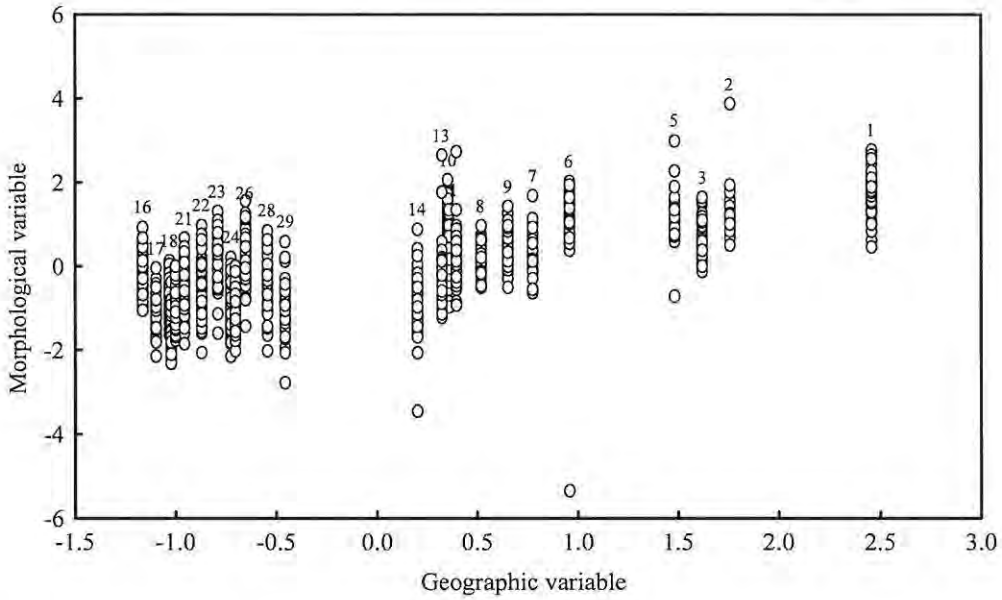


Figure 4.10: Canonical correlation plot for the first canonical variate of *S. serrata.*

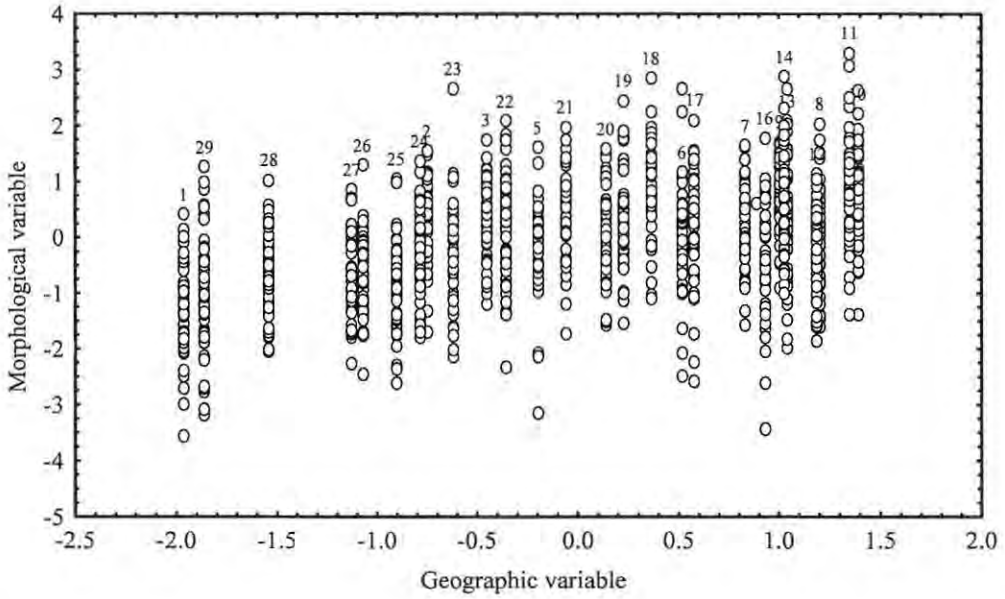


Figure 4.11: Canonical correlation plot for the second canonical variate of *S. serrata.*

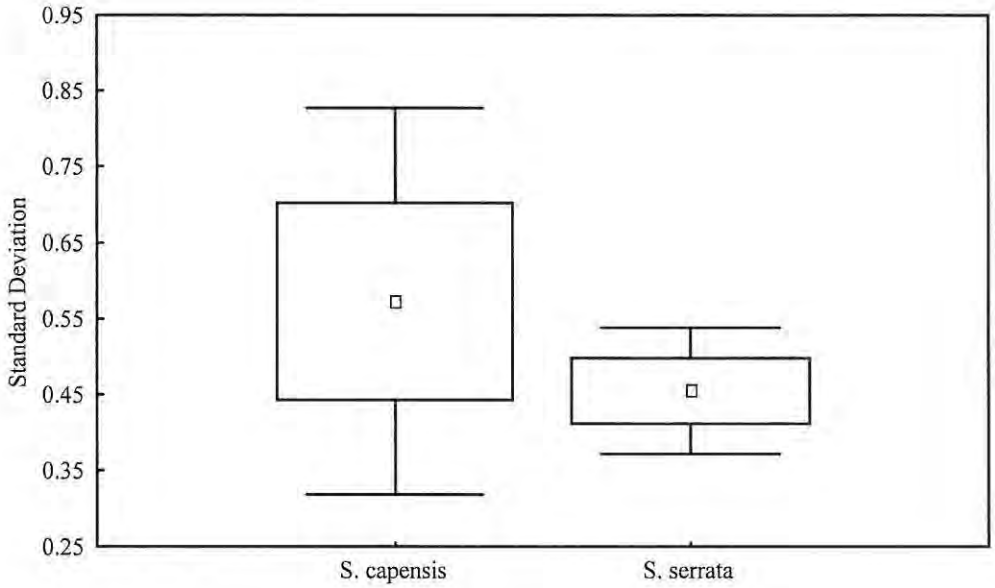


Figure 4.12: Box and whisker plot of individual variance in principal component 1 (size) of *S. capensis* and *S. serrata*.

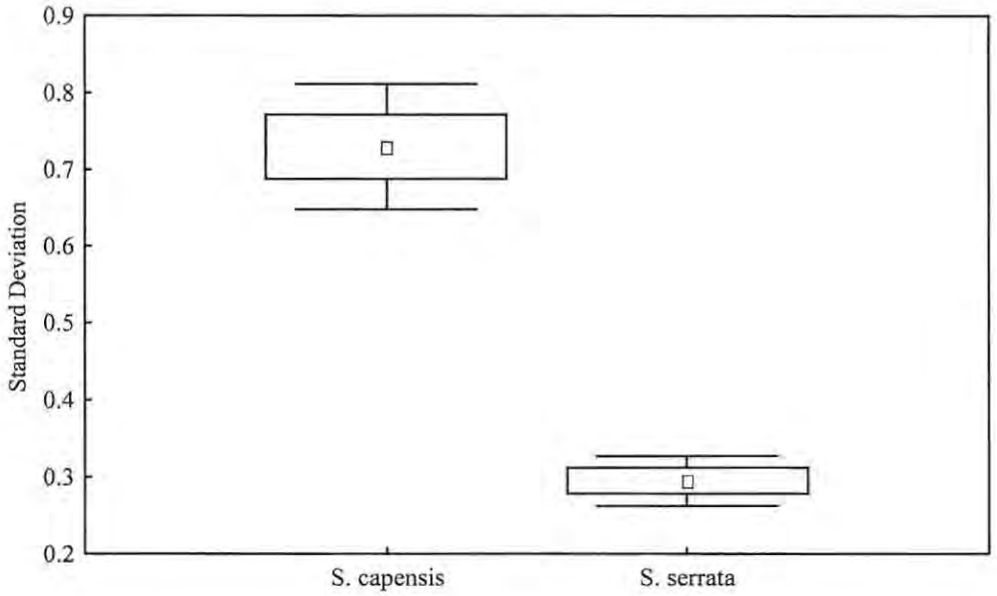


Figure 4.13: Box and whisker plot of individual variance in principal component 2 (shape) of *S. capensis* and *S. serrata*

CHAPTER 5: Total protein analysis

5.1 INTRODUCTION

Siphonaria capensis and *S. serrata* are con-generic and have different developmental modes. *Siphonaria capensis* is a pelagic developer. The larvae float in the ocean currents for at least one month before settling and metamorphosing into adults (Chambers and McQuaid, 1994b). While developing in the plankton, there is a high potential for these larvae to disperse great distances along the coast. Many species around the world have been found to produce larvae, which disperse in this way. These include: several littorinid snails (Janson, 1987; Johannesson, 1992; De Wolf *et al.*, 1998b; Johnson and Black, 1998), patellid limpets (Hurst and Skibinski, 1995; Ridgway *et al.*, 1998) and mussel species (Levinton and Suchanek, 1978; Grant *et al.*, 1992). Some species, notably a starfish, *Linckia laevigata* (Williams and Benzie, 1996), a mussel species, *Mytilus galloprovincialis* (Quesada *et al.*, 1995), a sea urchin, *Echinothrix diadema* (Lessios *et al.*, 1998) and a large aspidochirotide holothurian, *Holothuria nobilis* (Uthicke and Benzie, 2000) have larvae which travel thousands of kilometres before settling.

Siphonaria serrata is a direct developer. The adults lay egg masses in which larvae develop and hatch as completely metamorphosed juveniles (Chambers and McQuaid,

1994b). This mode of development should severely limit the potential to disperse. Once again, this has been found to be true in several species around the world including: European *Littorina* snails (Janson and Ward, 1984; Janson, 1987; Knight and Ward, 1991; Johannesson, 1992), a southern African whelk (Grant and da Silva-Tatley, 1997) and a South American sea anemone (Russo *et al.*, 1994).

The different dispersal potentials of these two *Siphonaria* species are expected to have a profound effect on levels of genetic variation. As discussed in Chapter 2, the high dispersal potential of *S. capensis* should result in high within-population variation. Among-population genetic variation in *S. capensis* is expected to be low due to high levels of gene flow, achieved through larval dispersal. The low dispersal potential of *S. serrata* should result in low within-population variation. Among-population genetic variation is expected to be high as populations of *S. serrata* will be relatively isolated from one another, due to limited genetic exchange.

Levels of larval dispersal are known to be influenced by ocean currents (Behrens Yamada, 1977; Vermeij, 1987; Bertness and Gaines, 1993; Kojima *et al.*, 1997; Brierley and Brandon, 1999; Hilbish *et al.*, 2002). Along the Cape coast of South Africa, the Agulhas Current is far off shore (approximately 270km at its furthest position; Figure 2.2; Chapter 2). The inshore currents in this coastal area have low velocities, are largely wind driven and vary greatly in direction (Harris, 1978; Schumann *et al.*, 1988). These weak, variable inshore currents may lead to a reduced level of larval dispersal in *S. capensis*. In the western region of the Cape coast, the Agulhas Current encounters the Benguela Current, which flows northward from the Cape of Good Hope (Duncombe Rae, 1991). Cape Agulhas is considered the most appropriate boundary between the Benguela

and Agulhas systems (Brown, 1992; Emanuel *et al.*, 1992; Largier *et al.*, 1992) and limited dispersal across this boundary is expected. The possible reduction in pelagic larval dispersal will, in turn, result in lower than expected levels of gene flow among populations of *S. capensis* on either side of Cape Agulhas.

As discussed in Chapter 3, there are several indirect techniques available for examining an hypothesis such as this. Polyacrylamide gel electrophoresis (PAGE) of total soluble proteins is not a widely utilized technique, but it is simple, quick and inexpensive (Nxomani *et al.*, 1994; Chambers *et al.*, 1996; Brown, 1999) and can be a useful indicator of genetic variation within a species (Burton, 1983). Unlike most genetic techniques, PAGE of total proteins can reveal several loci on a single gel (De Vienne *et al.*, 1996). Previously, this technique has been used to distinguish between bacteria strains (Kersters and De Ley, 1975; Swings *et al.*, 1976; Vera Cruz *et al.*, 1984; Jackman and Pelczynska, 1986; Andersen *et al.*, 1987), in population studies of *Tilapia* fish (Nxomani *et al.*, 1994) and various plants, including maritime pine (Petit *et al.*, 1995; De Vienne *et al.*, 1996), maize, barley, peas (De Vienne *et al.*, 1996) and proteas (Brown, 1999). This technique was also used to examine genetic diversity of seven species of *Siphonaria* limpets (Chambers *et al.*, 1996) including the two species currently being examined.

The aim of this study was to examine within- and among-population variation in *S. capensis* and *S. serrata* using PAGE of total soluble proteins.

5.2 METHODS

Samples of *S. capensis* and *S. serrata* were collected from 8 and 9 sites, respectively, along the South African Cape coast (*S. capensis*: sites 3, 5, 6, 8, 11, 12, 13, 15; *S. serrata*: sites 3, 5, 6, 7, 8, 10, 11, 12, 13 shown in Figure 2.1, Chapter 2). The limpets were collected as close to the low water mark as possible during a spring tide, within an area of approximately $1m^2$ at each site. The specimens were immediately frozen in liquid nitrogen and later stored at -40°C until protein analysis was carried out. Ten individuals of each species from each site were used for this analysis (except in Hermanus (6) where only three *S. capensis* individuals were found) and these were then treated as a population.

5.2.1 Extractions

Firstly, trials were carried out to establish the optimum method for extracting proteins from the foot muscle of each limpet. These trials included varying the extraction buffer used and time of boiling the samples. Two extraction buffers were tested: 0.1M phosphate buffer (pH 7.0) and 10mM Tris HCl (pH 7.5) with 1mM ethylenediaminetetraacetic acid (EDTA) and 1% *b*-mercaptoethanol. The Tris HCl (pH7.5) buffer containing EDTA and *b*-mercaptoethanol was found to be most useful in providing clear, discrete bands of protein, once these had been separated on a polyacrylamide gel. Variations in boiling times were also carried out, the optimum proved to be 5 minutes. The following extraction method was then utilized. Each specimen was removed from its shell and the gut and reproductive organs removed. The foot muscle was then washed in distilled water. These steps served to remove any parasites, which may have contaminated the final protein extract (Crossland *et al.*, 1993;

Hodgson *et al.*, 1993). Each specimen was then ground separately in liquid nitrogen with a mortar and pestle. One millilitre of extraction buffer (10mM Tris HCl (pH 7.5) containing 1mM EDTA and 1% *b*-mercaptoethanol) was added. Following centrifugation at 10 000rpm for 5 minutes, the supernatant was removed. Sample buffer of 125mM Tris HCl (pH 6.8), 2% *b*-mercaptoethanol, 4% sodium dodecylsulphate (SDS), 0.1% bromophenol blue and 20% glycerol was added in a ratio of 1:2 to the supernatant. Samples were boiled for 5 minutes and then stored at 4°C until electrophoresis was carried out.

5.2.2 Electrophoresis

Protein samples were loaded onto 10% discontinuous polyacrylamide gels. The gels contained SDS, which acted to partially denature and stabilize the proteins. A continuous buffer system of 0.02M Tris HCl, 0.2M glycine and 0.1% SDS was used during electrophoresis, which was carried out at 150V. Calibration proteins (Combithek, Boehringer Mannheim) were used to allow for gel to gel comparisons. Following electrophoresis, the gels were stained using Coomassie Brilliant Blue G-250. Destaining was carried out using 5% methanol, until a clear background with dark protein bands was achieved. Figure 5.1 shows examples of polyacrylamide gels run for both *S. capensis* and *S. serrata.*

5.2.3 Data Analysis

A total of 29 bands for *S. capensis* and 27 for *S. serrata* were scored by eye as either present or absent. Bands with low molecular weights were excluded and only bands of between 340kDa and 14kDa were scored.

Numerical Taxonomy and multivariate analysis SYStem (NTSYS) Version 2 was used to generate a similarity matrix, using a simple matching coefficient (m/n; where m = number of matched bands and n = total number of bands). The unweighted pair-group method using arithmetic averages (UPGMA) was used to generate dendrograms from the similarity matrix.

An Analysis of MOlecular VAriance (AMOVA) was performed using the software program ARLEQUIN (Schneider *et al.*, 1997). ARLEQUIN reads the total protein data as RFLP data, as such data are represented by presence and absence data and are regarded as single locus haplotypes within the ARLEQUIN program. AMOVA is identical to ANOVA mathematically (Peakall *et al.*, 1995) and was used as a nested ANOVA with populations nested into groups, to estimate the variance among individuals within populations, among populations and among groups of populations within each species. As well as calculating an AMOVA, the ARELQUIN package also calculated pairwise genetic distance values among populations. These were then used to generate dendrograms using the Unweighted pair-group method using arithmetic averages (UPGMA). A Wilcoxon matched pairs test was carried out to establish whether a significant difference occurred between the genetic distance values of each species. The genetic distances among populations of both species were also correlated to geographic distance between populations using Pearson's Product Moment coefficient.

5.3 RESULTS

Ten haplotypes were found within both *S. capensis* and *S. serrata*. These haplotypes were represented by an average of 25.15 bands per individual for *S. capensis* and 25.12 bands per individual for *S. serrata*.

5.3.1 *Siphonaria capensis*

A dendrogram generated by UPGMA (Figure 5.2) showed a range of similarity values for *S. capensis* from 1.00 to 0.64. Nineteen individuals showed no difference from one another. Most of these individuals were identical to others from the same population (Sea Point (3), Struisbaai (11), Mossel Bay (15) and Arniston (12)). However, there were two cases where members of different populations were identical. Two individuals from Witsand (13) were identical to an individual from Struisbaai (11) and one individual from each of the following populations, Arniston (12), Muizenberg (5) and Pearly Beach (8) were identical. Within-population variation appears to be high in *S. capensis* as individuals from particular populations formed mixed groupings rather than clustering together with individuals from their population of origin. The AMOVA confirmed a high level of within-population variation in *S. capensis*. Within-population variation was responsible for 66.4% of total variation (Table 5.1).

Pairwise genetic distance values (Table 5.2) among populations of *S. capensis* ranged from 0.047 (between populations 12 and 13) to 0.571 (between populations 3 and 11). These values are generally low and suggest that a high level of gene flow occurs among populations. These values are better illustrated as a dendrogram (Figure 5.3). Populations closest to one another geographically did not necessarily link directly to one another

within the dendrogram. This suggests that a large amount of gene flow may occur among the populations, except in the case of Sea Point (3) which remained separate from all the other populations at a linkage distance of 0.48 (Figure 5.3). The genetic distance values (Table 5.2) did not correlate significantly with geographic distance between populations ($r = 0.28$; $p > 0.05$). In comparison to the levels of within population variation, a low level of among-population variation was confirmed by the AMOVA. This AMOVA showed that among-population variation accounted for 32.5% of the total variation found in *S. capensis* (Table 5.1).

When the populations were split into two groups on either side of Cape Agulhas (sites 3, 5, 6, 8 and 11, 12, 13, 15), a very low level of genetic variation was found between them (1.16%; Table 5.1). This suggests that Cape Agulhas does not act as a barrier and does not have a strong limiting effect on larval dispersal in *S. capensis*.

5.3.2 *Siphonaria serrata*

A dendrogram generated by UPGMA for *S. serrata* (Figure 5.4), showed that similarity values ranged between 1.00 and 0.69. Fifty-five individuals of *S. serrata* showed no difference between themselves and at least one other individual from their population of origin. Clusters of individuals within the dendrogram showing no differences among them were not exclusively from a single population (Figure 5.4). Populations showing the greatest number of individuals with no variation amongst them included, Muizenberg (5) (7 individuals), Struisbaai (11) (6 individuals) and Witsand (13) (8 individuals). This dendrogram suggests that, as hypothesised, there was low within-population variation in *S. serrata* due to the low dispersal potential. However contrary to this, an AMOVA showed that, as for *S. capensis*, the bulk of the total variation in *S. serrata* (66.5%) was

still within-population variation (Table 5.3).

Pairwise genetic distance values among populations ranged from 0.00 (between populations 12 and 13) to 0.63 (between populations 5 and 6) in *S. serrata* (Table 5.4). These genetic distances show the relationship between populations more clearly when examined as a UPGMA dendrogram (Figure 5.5). There were two main groups of populations with Gansbaai (7), Hermanus (6) and Sea Point (3), all west of Cape Agulhas, grouping separately from the remainder of the populations (Figure 5.5). Surprisingly, the genetic linkage between the populations of Struisbaai (11) and Arniston (12) was zero, suggesting no genetic difference between these two populations. The genetic distance values (Table 5.4) among populations of *S. serrata* were found to be significantly correlated to geographic distance ($r = 0.43$; $p < 0.05$) suggesting that there is some isolation by distance between populations.

Although genetic distance between populations correlates significantly with geographic distance between populations, very little variation was found between the two groups of populations found on either side of Cape Agulhas (3.46%; Table 5.3).

5.3.3. Comparison between species

Contrary to expectations, a number of similarities in levels of genetic variation were found between *S. capensis* and *S. serrata*. It was unexpected that *S. capensis* and *S. serrata*, with differing developmental modes, would show similar results.

A greater number of identical individuals were seen in *S. serrata* (55 individuals) than in *S. capensis* (19 individuals) (Figures 5.2 and 5.4). This suggests that, as expected, *S.*

serrata had lower levels of genetic variation within populations than *S. capensis*. However, the AMOVAs illustrated strong similarities between the two species in the amount of within-population variation (Tables 5.1 and 5.3). *Siphonaria capensis* was expected to show high within-population variation, due to the high dispersal potential of the pelagic larvae. Unexpectedly, *S. serrata* also showed high within-population variation (Table 5.3). Due to the low dispersal potential of this species relatives should remain close to one another and within-population variation should be high.

Both species also showed similar levels of among-population variation (Tables 5.1 and 5.3). Again, this low level (relative to the within-population variation) of among-population variation was expected for *S. capensis* but not for *S. serrata*. The pairwise genetic distance values calculated for each species confirm this similarity between the two species as when compared, using a Wilcoxon matched pairs test ($T = 98.00$; $Z = 6.08$; $p > 0.01$), no significant difference was found between mean genetic distances.

Siphonaria capensis and *S. serrata* showed very low levels of between-group variation (Tables 5.1 and 5.3). This suggests that neither *S. capensis* nor *S. serrata* had strong genetic structuring on a large scale and also that Cape Agulhas did not form a strong barrier to dispersal. However, the pairwise genetic distance values for *S. serrata* were found to be significantly correlated to geographic distance along the coast. This suggests some isolation of populations and reduced dispersal potential within *S. serrata* in comparison to *S. capensis*, which showed no such correlation.

5.4 DISCUSSION

A study by Chambers *et al.* (1996) showed that, within the genus *Siphonaria*, higher genetic variability levels were associated with planktotrophic developers than with direct developers. The present study has not reflected a dramatically strong difference in genetic variation between *S. capensis* (planktotrophic developer) and *S. serrata* (direct developer). Levels of within-population variation were found to be high in both species (Tables 5.1 and 5.3). In comparison to the within-population variation, among-population variation was found to be low in both species (Tables 5.1 and 5.3) and genetic distance values did not differ significantly between the two species. While high within- and low among-population variation were expected in *S. capensis* (due to its high dispersal potential), it was anticipated that *S. serrata* would have low within-population variation and high among-population variation due to its limited dispersal potential. One important difference between the two species was found, i.e. genetic distance values between populations of *S. serrata* were significantly correlated to geographic distance, which was not the case for *S. capensis*. This suggests that, on a large scale, there is some genetic variation among populations of *S. serrata* and this may be due to a lower dispersal potential in this species than in *S. capensis*.

Several studies have found that the hydrography of a region influences genetic variation in a species, particularly those with pelagic larvae (Scheltema, 1971; Schaeffer *et al.*, 1985; Hunt, 1993; Kojima *et al.*, 1997). The low levels of among-population variation in both *Siphonaria* species suggest that the potential barrier at Cape Agulhas does not limit dispersal. Indeed, anchovy larvae were found to disperse widely in this area of the coast (Largier *et al.*, 1992), though of course they are more motile than larvae of *S. capensis*.

The similar levels of genetic variation both within and among populations of *S. capensis* and *S. serrata* may be caused by:

1. selection
2. similar levels of dispersal
3. lack of strong genetic influence on total soluble proteins, through environmental factors or developmental changes in protein expression causing similarity in proteins produced (Burton, 1983)
4. little genetic variation within the genes coding for the total soluble proteins within these two species.

If selection was the cause of the similar results between *S. capensis* and *S. serrata*, it should result in high genetic variation along the coast as individuals would become adapted to local conditions within the different populations (Slatkin, 1987). Hence, with the high level of within-population variation and low level of among-population variation seen in both species, it is extremely unlikely that selection is the main cause of the similar levels of variation between the two species.

It is possible that the similar levels of variation in these two species may result from similar dispersal rates. This would require either the dispersal of *S. capensis* larvae to be dramatically reduced or for *S. serrata* to have a higher than expected dispersal potential. Considering the low differentiation among populations and groups of populations, as compared to the differentiation within populations, as well as the lack of significant correlation between genetic distance and geographic distance, it is unlikely that larval dispersal of *S. capensis* is limited. Indeed, the inshore countercurrents of the Agulhas

Current along the Cape south coast are known to transport anchovy larvae in this area (Largier *et al.*, 1992) and so are likely to facilitate dispersal of *S. capensis* larvae too. While dispersal of the pelagic larvae of *S. capensis* is probably not limited, there is a chance that alternative dispersal strategies may enhance the dispersal of *S. serrata*. It is possible that egg masses or adults of this species disperse (Scheltema, 1975; Martel and Chia, 1991; Tatarenkov and Johannesson, 1994; Hummel *et al.*, 1997; Ruckelshaus, 1998). Such dispersal has been found in several direct developing species, including brooding bivalves and unidentified juvenile limpets (O'Foighil, 1989; Martel and Chia, 1991). Generally, however, these alternative dispersal events are considered rare and probably do not occur with great enough frequency to result in the very high levels of genetic similarity seen in *S. serrata* in this PAGE analysis (Hellberg, 1995). The fact that genetic distance values among populations of *S. serrata* were significantly correlated with geographic distance does suggest that dispersal of this species is, to some extent, more limited than that of *S. capensis*.

The apparent similarity of genetic differentiation found within both species could be due to environmental factors overriding any genetic influence on the soluble proteins of these species. Environmental factors, such as wave exposure (Newkirk and Doyle, 1975; Smith, 1981; Grahame and Mill, 1992; Hobday, 1995) and sea temperature (Boulding *et al.*, 1993) may influence the proteins or type of proteins expressed in the individuals, although these factors have not been shown to affect allozyme patterns. Alternatively, there may be developmental changes in protein expression (Burton, 1983).

The lack of variation in total soluble proteins of *S. capensis* and *S. serrata*, particularly among populations, could also be due to the lack of genetic variation within the genes

coding for these proteins. Chambers *et al.* (1996) concluded that genetic variation in this genus is small. Certainly, this study supports this idea.

PAGE of total soluble proteins has been suggested as an alternative to other protein based genetic techniques, namely allozymes (Nxomani *et al.*, 1994; Chambers *et al.*, 1996). It is an inexpensive, quick technique and has been useful for some population genetic studies such as *Tilapia* fish (Nxomani *et al.*, 1994), in the differentiation of subspecies of the protea complex, *Leucaendron elimense* (Brown, 1999), as well as in a study of variation on a species level in the genus *Siphonaria* (Chambers *et al.*, 1996). However, in terms of showing differences in genetic variation between *S. capensis* and *S. serrata*, it has not been a resounding success. Clearly, it does not reveal the detail of the genetic variation found when examining the same hypothesis with allozyme analysis (Chapter 6). This may be, in part, due to the lack of a strong genetic influence on total soluble proteins (Burton, 1983). PAGE may prove more useful for population studies when used in two dimensions, where the proteins are separated both by charge and molecular weight as found by Petit *et al.* (1995) and De Vienne *et al.* (1996).

5.5 CONCLUSION

PAGE analysis revealed little genetic variation in either *Siphonaria* species. Both species were found to show high levels of within-population variation and low levels of among-population variation. The only major difference between the two species, lay in the fact that genetic distance values correlated significantly with geographic distance in *S. serrata* only. All in all, PAGE appears to be useful only as a crude measurement of differentiation within a species (Brown, 1999).

Table 5.1: AMOVA of among-group (populations grouped east and west of Cape Agulhas), among-population and within-population variation in *S. capensis*.

Source of variation	df	Sum of squares	Variance components	% total variance	p
Among groups	1	10.563	0.029	1.16	>0.05
Among populations	6	53.580	0.813	32.48	<0.001
Within populations	65	107.967	1.661	66.35	<0.001
Total	72	172.110	2.503	100.00	

Table 5.2: Pairwise genetic distance values among populations of *S. capensis*.

Popn	3	5	6	8	11	12	13	15
3	-----							
5	0.566	-----						
6	0.464	0.198	-----					
8	0.410	0.240	0.269	-----				
11	0.571	0.361	0.303	0.423	-----			
12	0.385	0.323	0.283	0.171	0.211	-----		
13	0.440	0.204	0.236	0.163	0.266	0.047	-----	
15	0.554	0.135	0.197	0.241	0.413	0.258	0.233	-----

Table 5.3: AMOVA of among-group (populations grouped east and west of Cape Agulhas), among-population and within-population variation in *S. serrata*.

Source of variation	df	Sum of squares	Variance components	% total variance	p
Among groups	1	8.638	0.058	3.46	>0.05
Among populations	7	44.183	0.521	31.01	<0.001
Within populations	81	89.200	1.101	65.53	<0.001
Total	89	142.022	1.680	100.00	

Table 5.4: Pairwise genetic distance values among populations of *S. serrata*.

Popn	3	5	6	7	8	10	11	12	13
3	-----								
5	0.516	-----							
6	0.208	0.627	-----						
7	0.103	0.431	0.076	-----					
8	0.382	0.435	0.441	0.334	-----				
10	0.376	0.312	0.362	0.299	0.250	-----			
11	0.458	0.444	0.493	0.376	0.264	0.216	-----		
12	0.316	0.255	0.389	0.281	0.192	0.167	0.000	-----	
13	0.475	0.488	0.525	0.405	0.256	0.271	0.049	0.000	-----

S. capensis

S. serrata

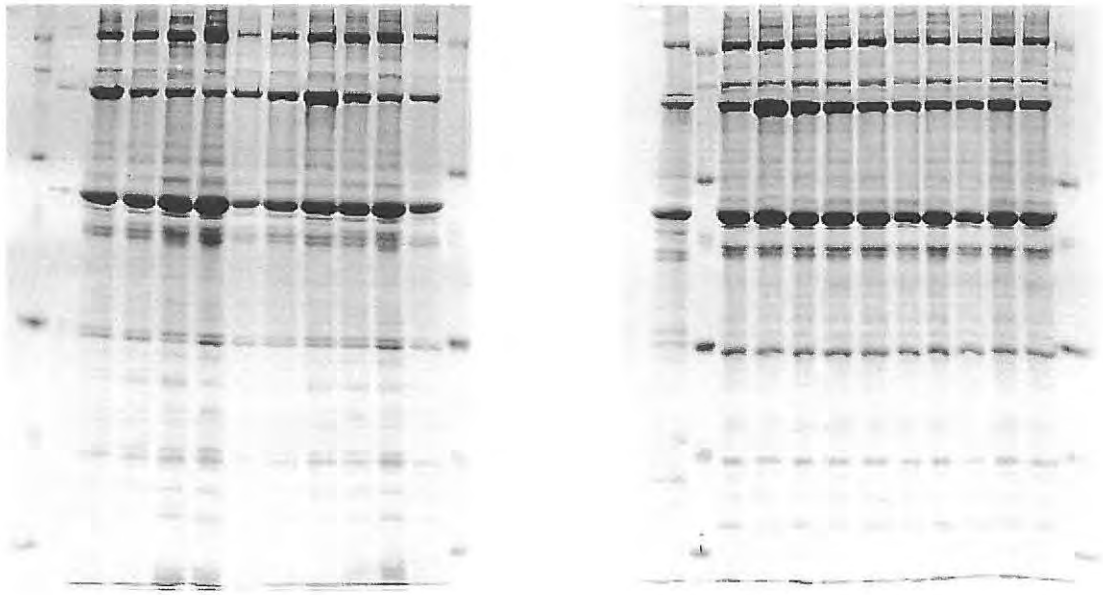


Figure 5.1: Examples of polyacrylamide gels used to separate the total soluble proteins of *S. capensis* and *S. serrata*.

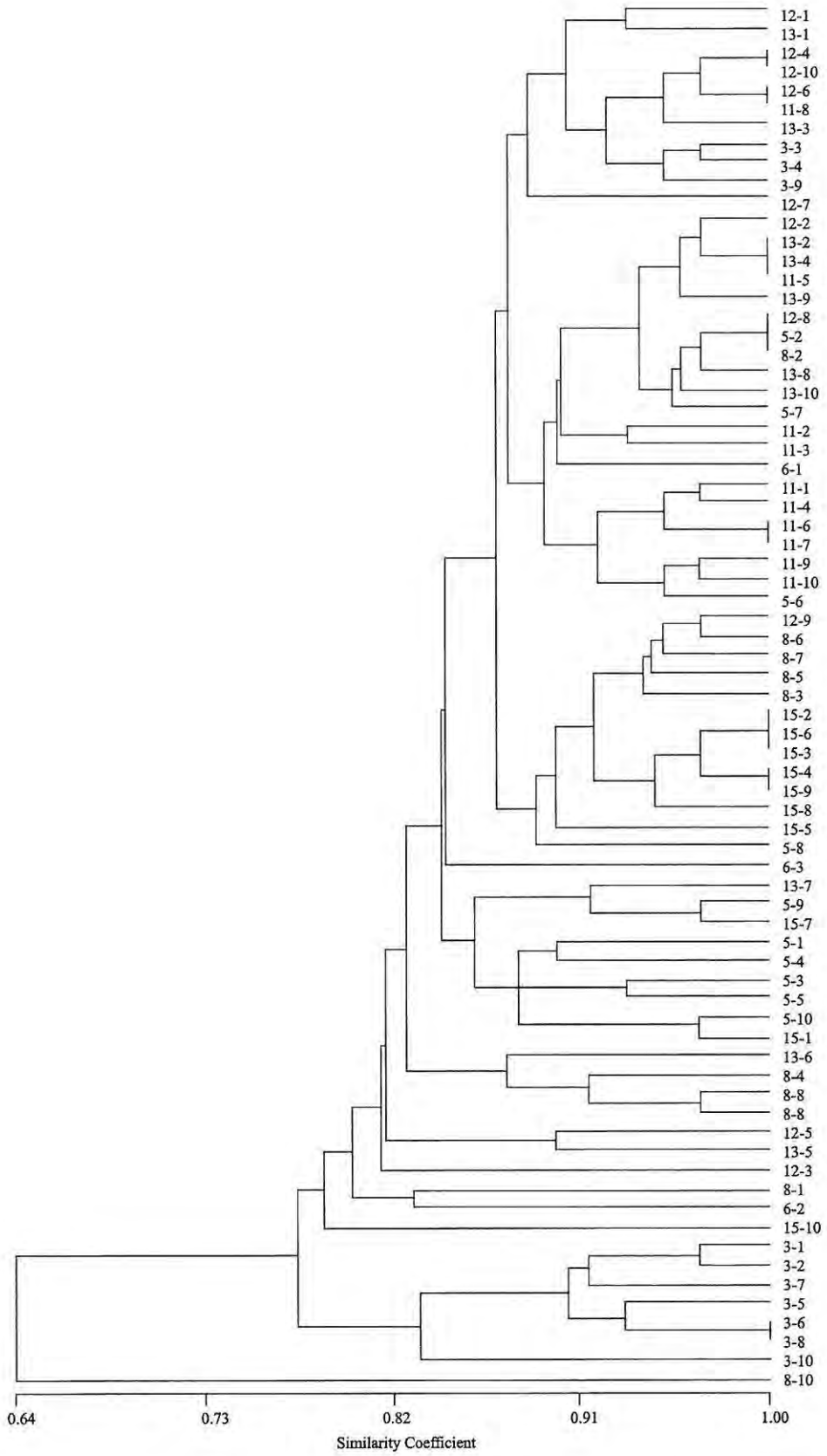


Figure 5.2: Dendrogram of *S. capensis* individuals generated using UPGMA and based on simple matches coefficient. Each individual is labelled according to the population to which it belongs and is then given an unique number.

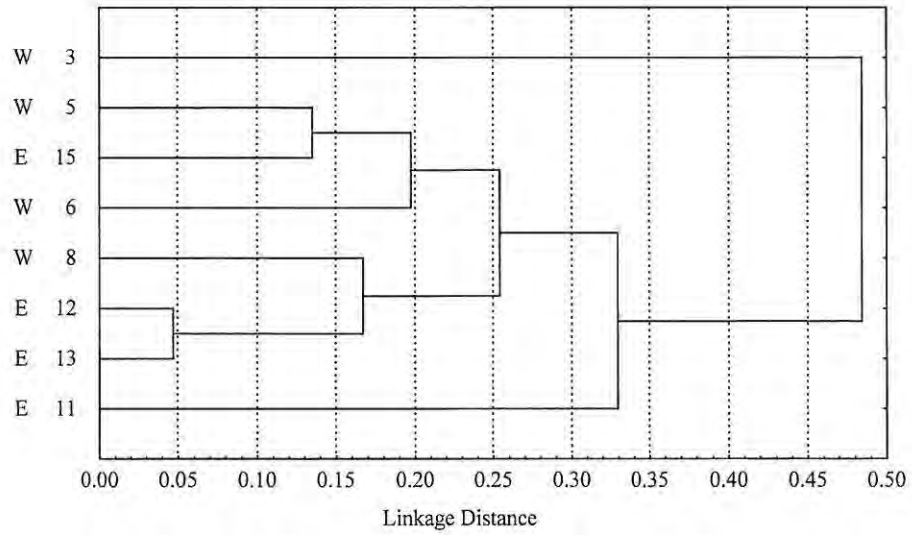


Figure 5.3: Dendrogram of *S. capensis* populations generated using UPGMA and based on pairwise genetic distance values. Populations labeled according to site number and position with respect to Cape Agulhas, i.e. west (W) or east (E) of Cape Agulhas.

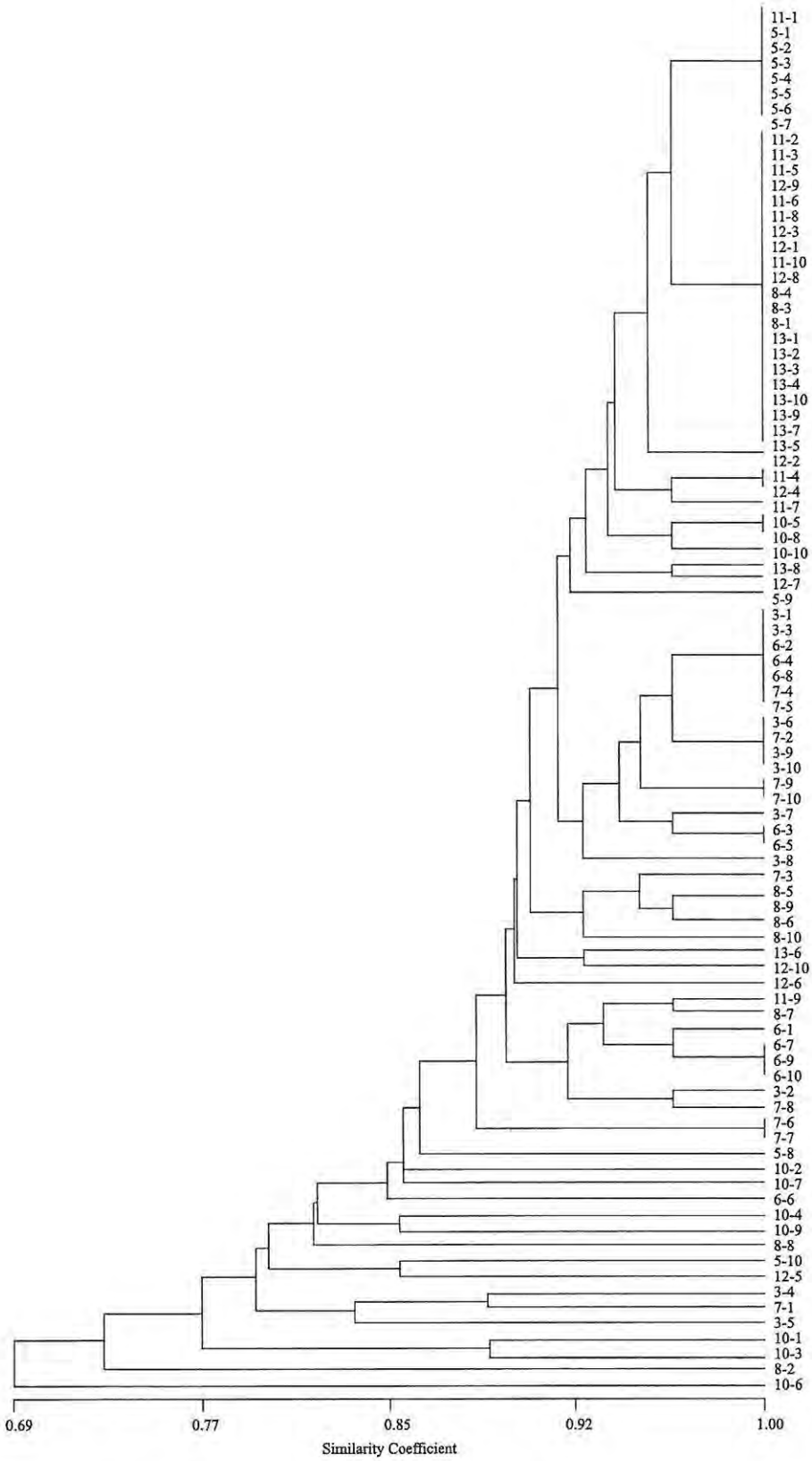


Figure 5.4: Dendrogram of *S. serrata* individuals generated using UPGMA and based on simple matches coefficient. Each individual is labelled according to the population to which it belongs and is then given an unique number.

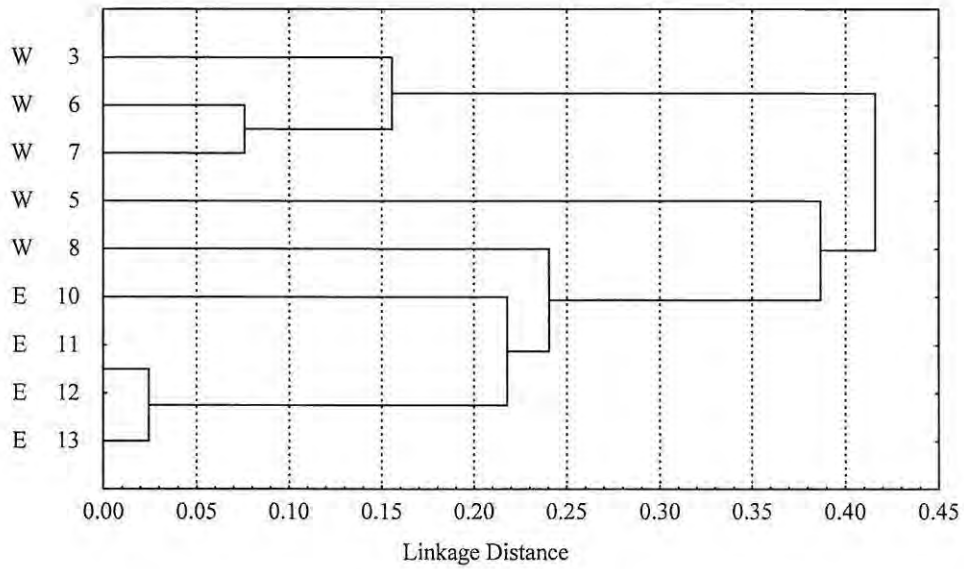


Figure 5.5: Dendrogram of *S. serrata* populations generated using UPGMA and based on pairwise genetic distance values. Populations labeled according to site number and position with respect to Cape Agulhas, i.e. west (W) or east (E) of Cape Agulhas.

CHAPTER 6: Allozyme analysis

6.1 INTRODUCTION

In marine benthic invertebrate populations it is assumed that high dispersal rates (usually achieved through the presence of a pelagic larval stage) result in high levels of gene flow and hence high genetic homogeneity among populations, even if they are widely separated. Contrary to this, species with low dispersal potential are expected to have low gene flow and high genetic differentiation among populations. Marine molluscs show strong support for assumptions made in this regard. Such links are seen clearly in the extensively studied periwinkle snails of the Littorinidae, on a wide variety of scales (Berger, 1973; Snyder and Gooch, 1973; Wilkins and O'Regon, 1980; Janson and Ward, 1984; Johannesson, 1988; Johannesson and Johannesson, 1989; Ward, 1990; Liu *et al.*, 1991; Tatarenkov and Johannesson, 1994; Johnson and Black, 1998 and 1999; Kyle and Boulding, 2000).

Other marine invertebrate species conform to predictions that with high levels of dispersal come high levels of gene flow (Grassle and Grassle, 1978; Slatkin, 1985). Through allozyme analysis the following planktonic developing species were found to exhibit high levels of gene flow: the Queen scallop, *Chlamys opercularis* in the northern Irish sea (Macloed *et al.*, 1985); the Queen conch, *Strombus gigas* in the

Caribbean (Mitton *et al.*, 1989); the South American clam, *Venus antiqua* (Gallardo *et al.*, 1998); two planktonic chaetognaths, *Parasagitta elegans* and *Eukrohnia hamata* (Thuesen *et al.*, 1993); the sea urchin, *Echinometra mathaei* in Western Australia (Watts *et al.*, 1990); a starfish, *Linckia laevigata* in the southern Pacific Ocean (Williams and Benzie, 1996); brown mussels, *Perna perna* along the southern African coast (Grant *et al.*, 1992); surf bivalves, *Donax deltoides* along the Eastern Australian coast (Murray-Jones and Ayre, 1997); the bivalve *Quadrula quadrula* (Berg *et al.*, 1998); southern oyster drill, *Stramonita haemostoma* (Liu *et al.*, 1991); limpets, *Patella vulgata* along the coast of the British Isles (Hurst and Skibinski, 1995) and *Siphonaria* sp. (Johnson and Black, 1984b) and *S. kurraacheensis* (Johnson *et al.*, 2001) in Western and South Australia, as well as an hydrothermal vent dwelling archaeogastropod species (Craddock *et al.*, 1997).

"Gene flow is presumably limited by direct development in benthic egg capsules" (Grant and Utter, 1987). This is seen clearly through allozyme studies of the following direct developing species: a copepod, *Tigriopus californicus* along the Californian coast (Burton *et al.*, 1979); a whelk, *Nucella lamellosa* (Grant and Utter, 1987); the Atlantic octopus, *Pareledone turqueti* (Allcock *et al.*, 1997); and a philopatric black coral *Antipathes fiordensis* (Miller, 1998).

Comparative studies of species exhibiting differing developmental modes also support these ideas on the link between gene flow and level of dispersal. Such studies include: two intertidal starfish, *Patiriella calcar* and *P. exigua* (Hunt, 1993); two sponge-dwelling shrimps (*Synalpheus brooksi* and *S. pectintger*) (Duffy, 1993); two solitary corals (*Balanophyllia elegans* and *Paracyathus stearnsii*) (Hellberg, 1996)

and tropical sea anemones (*Bunodosoma caissarum* and *Actinia bermudensis*) (Russo *et al.*, 1994).

However, not all researchers agree that a strong connection exists between pattern of reproduction and gene flow in marine invertebrates (Burton, 1983; Liu *et al.*, 1991; Tatarenkov, 1992; Palumbi, 1995; Grant and da Silva-Tatley, 1997). Some allozyme studies show that a variety of species with low dispersal potential have high genetic similarity among populations separated by long distances. Examples include: a direct developing whelk, *Bullia digitalis*, along the South African coast (Grant and da Silva-Tatley, 1997): a brooding coral, *Pocillopora damicornis*, among reefs up to 1200km apart along the Great Barrier Reef (Ayre *et al.*, 1997) and two amphipod species, *Talitrus saltator* and *Talorchestia deshayesii*, both lacking a larval stage (Bulnheim and Scholl, 1986). *Littorina saxatilis*, a direct developer, was found to colonize "new" habitats within two years, thus appearing to possess an unusually high dispersal potential, similar to that of a pelagic developer (Johannesson and Warmoes, 1990).

Some species with pelagic larvae, also fail to conform to the theory that gene flow may be determined by mode of reproduction. The pelagic larvae of alpheid shrimps (Knowlton and Keller, 1986), abalone (Prince *et al.*, 1987) and nudibranchs (Todd *et al.*, 1988) have been shown to settle extraordinarily close (few metres) to their parents. Two prawn species with planktonic larvae, *Palaemon adspersus* and *P. squilla* showed significant genetic heterogeneity among populations (Berglund and Lagercrantz, 1983), as did the calanoid copepod, *Labidocera aestiva* (Bucklin and Marcus 1985), a Mediterranean mussel *Mytilus galloprovincialis* along the Spanish coast (Quesada *et al.*, 1995), a scleractinian coral, *Mycedium elephantotus* (Dai *et al.*,

2000) and a holothurian, *Holothuria scabra* (Uthicke and Benzie, 2001). Finally, a corallivorous snail, *Drupella cornus*, showed marked genetic differentiation among groups less than 80m apart (Johnson *et al.*, 1993). These studies suggest that dispersal of larvae may be limited in some way (Bucklin and Marcus, 1985), possibly by an ecological barrier (Scheltema, 1975; Quesada *et al.*, 1995; Williams and Benzie, 1996; Johnson and Black, 1998). Alternatively, dispersal may occur but selection on populations may be so great that it overrides any effect of gene flow (Johnson and Black, 1982; Bucklin and Marcus, 1985; Noy *et al.*, 1987; Johannesson *et al.*, 1995). It is suggested that if differentiation between populations is purely adaptive, then both high and low dispersal species will become genetically variable due to differential selection in different habitats (Tatarenkov and Johannesson, 1994; Grant and da Silva-Tatley, 1997).

When examining the effect of gene flow on the genetic structure of any population or species, the study should ideally include the following features (Hellberg, 1996):

1. samples taken from the same localities
2. use of the same genetic markers
3. sister taxa differing only in their mode of reproduction.

The *Siphonaria* species used in this study do not conform to the suggestion that all genetic markers be common to both species, as different allozyme loci were found to be polymorphic. However, this study conforms to the suggestions that samples be taken from the same localities (see Figure 2.1, Chapter 2) and that sister taxa be used. These species differ only in the sub-genera to which they belong and in mode of reproduction (Chambers and McQuaid, 1994a). *Siphonaria capensis* is a

planktotrophic developer, while *S. serrata* is a direct developer (Chambers and McQuaid, 1994a; Hodgson, 1999). As discussed in Chapter 2, it is expected that the pelagic larvae of *S. capensis* will allow for extended dispersal of this species. This is predicted to lead to high genetic differentiation within populations. Likewise, low genetic differentiation (high similarity) should be found among *S. capensis* populations. On the other hand, direct development in *S. serrata* should result in limited dispersal and hence high homogeneity within populations. High genetic differentiation (low similarity) should occur among populations of *S. serrata*.

This study aimed to investigate the effect of reproductive mode on gene flow in *S. capensis* and *S. serrata*, using allozyme analysis. This technique detects differences in enzymes resulting from variation in the amino acid sequence within an enzyme structure. The amino acid sequence, in turn, is controlled by changes in the DNA. So each allozyme can be considered as a single locus genetic marker and can be reliably used to determine genetic differences among populations (Nei, 1972; Thuesen *et al.*, 1993).

Enzyme electrophoresis is a powerful tool (Eanes and Koehn, 1978) and has been extensively employed for genetic analysis of a great variety of species in the plant (Guries and Ledig, 1982) and animal kingdoms (Ward, 1990). Enzyme electrophoresis has been used to: construct phylogenies of closely related species (e.g. Chambers, 1978; Gutierrez *et al.*, 1983; Havenhand *et al.*, 1986; Boulding *et al.*, 1993; Wilson *et al.*, 1999); clarify sibling species status (e.g. Johnson *et al.*, 1977; Ward and Warwick, 1980; Janson, 1985b; Janson and Ward, 1985; Knight and Ward, 1991; Grahame *et al.*, 1992; Dillon and Lydeard, 1998; Ridgway *et al.*, 1998; Weber

et al., 1997; Johnson and Black, 1999; Jurss *et al.*, 1999); and study population differentiation (e.g. Eanes and Koehn, 1978; Ward and Warwick, 1980; Burton, 1983; Janson, 1985a; Crouau-Roy, 1988; Beaumont *et al.*, 1993; Njiokou *et al.*, 1994; Swart and Ferguson, 1997; Arnaud and Daguzan, 1999). Apart from having become a relatively cheap, reliable technique for genetic studies, enzyme electrophoresis has a very robust, well-researched set of statistical methods for analysing the raw data (e.g. Nei, 1972; Slatkin, 1987; Hartl and Clark, 1989). Since the 1960's this biochemical technique has given some surprising insights into the ecology and evolution of marine invertebrates. Much of this work has focused on the geographic scale of population differentiation (Burton, 1983). Of particular interest, in this study, is the usefulness of allozyme analysis in understanding population structure and genetic variation within the marine invertebrates, *S. capensis* and *S. serrata* along the coast of South Africa.

6.2 METHODS

Samples of *S. capensis* and *S. serrata* were collected from 11 and 12 sites, respectively, along the Cape coast and from the same 14 sites along the Transkei coast (detailed in Figure 2.1, Chapter 2). The specimens were immediately frozen in liquid nitrogen and later stored at -40°C for 6 months, until allozyme analysis was carried out. Twelve individuals of each species from each site were used for analysis and these were treated as a "population". These populations were later grouped together (as shown in Figure 6.1) to facilitate interpretation of the results and to reveal patterns of genetic variation and gene flow found along the coastline. This grouping structure was based on placing sites closest to one another in the same group and allowed for an increase in sample size. For a small-scale study, a further 40 individuals of *S. serrata* only were collected from each of

five populations (I to V) located east of Port Elizabeth, between Bushman's River Mouth and Hamburg (Figure 2.1; Chapter 2). These populations were positioned with increasing distance between them, populations I and II were 1km apart, II and III 5km apart, III and IV were 23 km apart and population V was 62km from population IV. Total distance between populations I and V was approximately 90 km.

6.2.1 Extractions

Firstly, trials were carried out to establish the optimum method for extracting proteins from the foot muscle. These trials included variations in time of sonification and centrifugation as well as a variety of extraction buffers. The following extraction buffers were tested: 0.15M Tris with 0.05M citrate (pH 7); 0.1M Tris HCl (pH 8); 0.13M Tris with 20% glycerol (pH 6.8); and finally 0.05M piperazine-N,N'-bis (2-ethanesulphonic acid) 1,4-piperazinediethanesulphonic acid (PIPES). The buffer containing 0.05M PIPES was found to be most useful in providing clear, discrete bands of protein once these had been separated on a gel. Several trials were then run using 1%, 3% and 5% Triton x100 and 1%, 3% and 5% 3-[(3-chloamidopropyl)dimethylammonio]-1-propane-sulphate (CHAPS) as detergents within the PIPES buffer. 5% Triton x100 was found to be the most successful detergent, again resulting in clear banding of the proteins. The following extraction method was then used to extract enzymes from individual limpets. Firstly, each specimen was removed from its shell. The gut was removed and the foot muscle was washed in distilled water to avoid contamination of the final protein extract by parasites (Hodgson *et al.*, 1993) and food from the gut. Each specimen was ground separately using a mortar and pestle. Extraction buffer of 0.05M PIPES containing 5% Triton x100 was added in proportion to the amount of tissue present, to give a similar (but unquantified) concentration of protein within each extract. Between 0.02g and 0.40g

of foot tissue was used from each specimen and the volume of buffer added ranged between 160µl and 650µl. Following sonification for 5 minutes, the samples were centrifuged at 4000rpm for 20 minutes at 4°C. The supernatant was then removed and stored for no longer than 24 hours before electrophoresis was carried out.

6.2.2 Electrophoresis

General electrophoretic conditions were taken from the protocols of Herbert and Beaton (1989) and others were adapted from Harris and Hopkinson (1976). Allozymes were separated using cellulose acetate gels at a constant voltage of 200V (2-15mA) for 20 or 30 minutes. Continuous buffer systems were used during electrophoresis, these being either 0.25M Tris with 2M glycine buffer (TG) or 0.4M N-(3-aminopropyl)-diethylamine buffer with 0.2M citric acid (CAEA) depending on the enzyme being examined (Herbert and Beaton, 1989). Staining procedures were adapted from Harris and Hopkinson (1976) and Herbert and Beaton (1989). Three millilitres of molten agar were added to the stain immediately before use, allowing a very small volume of staining mixture to be used with minimal dispersion of stained bands (Fulton *et al.*, 1992). Twenty-one enzymes were screened for polymorphisms. This ensured that as many loci as possible were sampled. The number of loci affects heterozygosity and genetic diversity estimates more severely than the number of individuals sampled (Gorman and Renzi, 1979). Table 6.1 shows the electrophoretic conditions used for each enzyme, as well as the Enzyme Commission (EC) Numbers. Enzyme nomenclature followed that of Harris and Hopkinson (1976), in line with the International Union of Biochemistry Nomenclature Committee (1984). Five and four of the loci screened were found to be polymorphic for *S. capensis* and *S. serrata*, respectively. Figure 6.2 shows a few examples of the cellulose acetate gels run for

both species. Noticeably, the resolution of the gels is relatively low. This was due to the large amount of mucopolysaccharides found within the tissue of both species. However, the resolution was sufficiently good to score the polymorphic loci accurately.

6.2.3 Data Analysis

Each polymorphic allozyme was scored for presence or absence of bands, for every individual. Departures from Hardy-Weinberg equilibrium were tested within each population, using the maximum likelihood G test (Sokal and Rohlf, 1981) with 1 degree of freedom. Gene diversity (H) was defined as the expected heterozygosity values within a population (Nei, 1978). A conservative, nonparametric Wilcoxon matched pairs test was carried out to establish whether significant differences occurred between the mean H values of each population of the two different species. Genetic differentiation among populations was calculated using Wright's fixation indices, F, (Hartl and Clark, 1989) as modified by Weir and Cockerham (1984). These indices included F_{IS}, an inbreeding coefficient within each population, F_{IT}, an inbreeding coefficient relative to the total collection of populations, and F_{ST}, a measure of the amount of allele frequency differentiation among subpopulations due to random drift (Crow & Kimura 1970). Single populations were then grouped together (as shown in Figure 6.1) to facilitate statistical interpretation of the results and to allow for more precise pairwise comparisons of genetic differentiation and gene flow within each species. These groupings were based on geographic proximity and contained approximately four populations each. Nm (Wright, 1969 cited in Hartl and Clark, 1989) was calculated from the F_{ST} values to give an indication of the amount of gene flow occurring among groups of populations. This approach assumes the island model of genetic structure. Both F_{ST} and Nm values within

each coastal region were compared using t-tests for dependent samples. Nei's standard genetic distance (D), which depends on differences in homozygosity (Nei, 1978), was also calculated, using Hillis' (1984) modification of Nei's (1972) method. The formulae used to carry out these analyses are given in Appendix I.

6.3 RESULTS

Polymorphic loci examined within both species were found to have a single prevalent allele. There was no significant deviation from Hardy-Weinberg equilibrium within any of the populations of either species, including the small scale study populations of *S. serrata*. In terms of the large scale study of both *S. capensis* and *S. serrata*, this must be regarded cautiously due to the small sample size within each population. This suggests that the populations, in general, satisfied the Hardy-Weinberg expectations in terms of random mating, absence of selection acting on the loci and immigration (Lessios, 1992). It also suggests that the polymorphisms present are genotypic in origin and are not due simply to biochemical effects (Swart *et al.,* 1994).

6.3.1 *Siphonaria capensis*

All five polymorphic loci showed variation throughout the sampling range and most populations showed high proportions of polymorphic loci, i.e. 3 to 5 polymorphic loci per population (Figure 6.3A). The exceptions were Cintsa (18), where only PGM was polymorphic and Saldahna (1), Sea Point (3) and Witsand (13) which showed polymorphisms in two of the loci (Figure 6.3A). Each locus was found to be polymorphic along sections of the coast except for AAT. This locus showed regional homogeneity along the western area of the Cape coast, from Saldahna (1) to

Muizenberg (5). Details of proportions of polymorphic loci are given in Appendix II.

Mean gene diversity (H) values (calculated from the polymorphic loci only) along the coast ranged from 0.033 at Sea Point (3) to 0.300 at Hamburg (16) (detailed in Appendix III). The mean H values along the Cape coast were found to range from 0.033 at Sea Point (3) to 0.183 at Mossel Bay (15) and Melkbosstrand (2) (Figure 6.3B). The Transkei coast had a wider range of mean H values ranging from 0.050 at Cintsa (18) to 0.300 at Hamburg (16). Most H values were greater than 0.1, except the Cintsa (18), Morgan's Bay (19) and Port Edward (29) populations (Figure 6.3B). Using a Mann-Whitney U test, the H values of the Cape and Transkei coastal regions were found not to differ significantly ($U = 58.50$; $Z = -0.753$; $p = 0.451$).

The overall F_{IS} value for *S. capensis* populations was 0.466 (Table 6.2). This is a very high value and suggests that some inbreeding may have occurred within populations. An elevated F_{IS} value, such as this, creates a concern as to the reliability of the Hardy-Weinburg equilibrium found to exist in this species. This disparity may be caused by the small sample sizes within each population. These high F_{IS} values contradict the relatively high levels of genetic variation seen in the proportions of polymorphic loci and gene diversity values (Figure 6.3A and B).

The mean F_{ST} value for *S. capensis* populations was found to be 0.25 (+/- 0.082) (Table 6.2), suggesting that there is great genetic differentiation among populations over the entire coast. (F_{ST} values ranging between 0.15 and 0.25 are said to indicate "great" genetic differentiation (Wright, 1978 cited in Todd *et al.*, 1994)). Genetic differentiation among populations of *S. capensis* within each of the two coastal

regions was found to be very consistent (F_{ST} = 0.228 ±0.090 along the Cape coast and 0.224 ±0.099 along the Transkei coast).

Pairwise comparisons of the grouped data revealed low F_{ST} indices (Table 6.3). Genetic differentiation (F_{ST}) differed significantly between the two coastal regions was for *S. capensis* (Mann Whitney U test: U = 0.00; Z = 1.964; $p < 0.05$). Low F_{ST} values were expected, due to the high dispersal potential of this species. Neighbouring groups of populations were expected to show low genetic differentiation from one another. This was indeed the case with neighbouring groups E and F (125km apart) showing little genetic differentiation (F_{ST} = 0.0054; Table 6.3). The groups A and F, occurring at opposite ends of the sampling range (approximately 1600km apart), showed a higher level of genetic differentiation (F_{ST} = 0.126; Table 6.3). These results showed a very clear link between F_{ST} and distance between paired groups of populations (Figure 6.4A). Pairwise F_{ST} values between single populations are given in Appendix IV.

Gene flow (Nm) was found to be low between coastal regions, as illustrated by groups A and F where Nm =1.729 (Table 6.3). However, Slatkin (1987) would consider this significant gene flow as he argued that at least one individual transported between two extreme groups per generation (Nm = 1) is sufficient to prevent strong genetic differentiation. High levels of gene flow were found between neighbouring groups within each coastal region, e.g. groups A and B, Nm = 3.460 and groups D and E, Nm = 15.217 (Table 6.3). Gene flow values were found to be significantly different (Mann Whitney U test: U = 0.00; Z = -1.964; $p < 0.05$) between the two coastal regions i.e. groups A, B and C versus groups D, E and F, with gene flow being

dramatically higher along the Transkei coast. The highest Nm value was 46.109 between E and F groups, from the Transkei coast (Table 6.3), while the highest value along the Cape coast was 3.800 (groups A and C; Table 6.3). Pairwise Nm values between each populations are given in Appendix IV.

A scatterplot of $\log_{10}$(Nm) versus distance along the entire coast for all pairs of population groups (Figure 6.4B) showed that Nm was negatively correlated with distance between groups of populations ($r = 0.67$, $p < 0.05$). Three pairs of population groups, namely A-B, A-C and B-C all along the Cape coast (indicated by hollow symbols) were found to deviate from the predicted linear relationship. These showed a lower than predicted level of gene flow. This departure from the predicted relationship illustrates a discontinuity but not a total absence of gene flow, as the Nm value between these groups was still higher than the conventional cut off point of Nm = 1. This may be a result of the oceanic processes occurring in the region. Broadly, the data suggest that an effective geographic distance of isolation for *S. capensis* was approximately 1600km, which is where Nm drops below 1.

A multiple regression was then carried out to further investigate the relationship between gene flow (dependent variable = $\log_{10}$(Nm)) and both the distance of the Agulhas Current from the shore and distance between groups of populations along the coast (independent variables). The distance of the Agulhas Current from the shore was estimated as the 500m isobath (C Duncombe Rae, *pers comm*), as the continental shelf plays a strong role in determining the path of the Agulhas Current (Duncombe Rae, 1991). The Agulhas Current, as discussed later, is thought to be responsible for influencing levels of gene flow in *S. capensis*, through its effects on larval dispersal.

A significant negative relationship was found to exist between gene flow and distance between groups of populations and distance of the Agulhas current from the coast (Regression equation: $y = -0.57(\pm 0.14)x_1 + -0.58(\pm 0.14)x_2 + 1.36$; where x_1 = distance of the Agulhas current from the shore and x_2 = distance between groups of populations along the coast. $r^2 = 0.73$; $F = 19.5$; $p < 0.05$).

Nei's genetic distance between populations was calculated and analysed by an UPGMA to produce a dendrogram (Figure 6.5). Most populations grouped together according to the coastal region to which they belong geographically. The Transkei populations formed two groups before linking to the Cape coast group. This supports the patterns of genetic differentiation among populations shown by the F_{ST} and gene flow results. Interestingly, the populations of Mossel Bay (15) and Stilbaai (14) linked more closely to populations of the Transkei region (some 500km away) than to those of the Cape region to which they belong geographically, where the nearest population is only 40km away. This link suggests that gene flow, facilitated by larval dispersal is occurring and further that it is probably being assisted by the Agulhas current flowing along the coast.

6.3.2 *Siphonaria serrata*

Within *S. serrata* populations, all four loci were polymorphic throughout the sampling range (Saldanha; 1 to Port Edward; 29) with the exception of G6PDH, which showed no polymorphisms north of Hamburg (16) along the Transkei coast (detailed in Appendix V). The NP locus also showed no polymorphisms along the northern Transkei coast, between Qolora Mouth (20) and Port Edward (29), with the exception of Mkambati (28) (Appendix V). A number of populations showed only a

single polymorphic locus. Hluleka (25), Coffee Bay (24), The Haven (22), Mazeppa (21), Witsand (13) and Arniston (12) showed polymorphism at the MDH locus, Die Dam (8) and Muizenberg (5) showed polymorphism in G6PDH and Cape Agulhas (10) was polymorphic for NP. It appears that MDH was a major contributor to the variation found along the Transkei coast and the eastern area of the Cape coastal region. No single *S. serrata* population showed polymorphisms within all four loci (Figure 6.3C).

Mean gene diversity values (H) along the entire coast ranged from 0.021 at Die Dam (9), Struisbaai (11) and Hamburg (16) to 0.275 at Sea Point (3), detailed in Appendix VI and illustrated by Figure 6.3D. Using a Mann-Whitney U test, the range of mean H values was found not to differ significantly between the Cape coast [0.021 at Die Dam (9) and Struisbaai (11) to 0.275 at Sea Point (3)] and the Transkei coast [0.021 at Hamburg (16) to 0.167 at Port St Johns (27)] ($U = 63.5$; $Z = -0.739$; $p = 0.46$).

The overall F_{IS} value for *S. serrata* populations was high at 0.323 (Table 6.4). This suggests that a substantial amount of inbreeding occurred within this species.

When the entire sample range was considered, F_{ST} was high in *S. serrata* (0.395 ± 0.28; Table 6.4). Along the Transkei coast, *S. serrata* populations showed a lower F_{ST} value (0.298 ± 0.11), than among populations along the Cape coast (0.486 ± 0.36). These F_{ST} values (i.e. >0.25) show "very great" genetic differentiation among populations (Wright, 1978 cited in Todd *et al.*, 1994).

Pairwise F_{ST} values among groups of *S. serrata* populations ranged from 0.008

(between groups A and B) and 0.150 (between B and C groups; Table 6.5). The pairwise F_{ST} values calculated for *S. serrata* were found to be erratic with no obvious relationship existing between F_{ST} and distance between groups of populations (Figure 6.4B). Groups furthest from one another did not necessarily show the greatest level of genetic differentiation. In fact, the results were often quite contrary to such a situation. Groups A and F had a pairwise F_{ST} value of 0.016, while groups A and C had a pairwise F_{ST} value of 0.125 (Table 6.5). No significant difference was found between the levels of genetic differentiation (F_{ST}) within the Cape and Transkei coastal regions (Mann Whitney U test: U = 2.00; Z = 0.577; p = 0.563). Pairwise F_{ST} values among single populations are given in Appendix VII.

Overall, the Nm values for *S. serrata* also appeared chaotic, with no distinct trends. The highest rate of gene flow was found to occur between groups A and B (Nm = 30.37), with the second highest rate occurring between the two most widely separated groups, namely groups A and F (Nm = 15.02; Table 6.5). Along the Cape coast, gene flow decreased rapidly, almost thirty times between groups A and B (Nm = 30.37) and groups B and C (Nm = 1.42; Table 6.5). Within the Transkei region, gene flow decreased more gently with distance, with the highest level of gene flow occurring between groups D and E (Nm = 11.91) and the lowest between groups D and F (Nm = 4.87; Table 6.5). As expected from these fairly erratic results, there was no difference in the gene flow values within the two coastal regions (Mann Whitney U test: U = 2.00; Z = -0.577; p = 0.563). Gene flow values within both coastal regions were all >1, indicating moderate levels of gene flow among all populations. When low levels of gene flow occur among populations it can be expected that the populations are fortuitously similar genetically, e.g. groups A and F. This is also expected when few

polymorphic loci are examined. So in the case of *S. serrata*, F_{ST} may indicate genetic similarity and the assumptions of the island model (upon which Nm values derived from F_{ST} is based) are violated and thus no clear statements can be made in regards to gene flow. Again, pairwise Nm values between single populations are given in Appendix VII.

Nm was not significantly correlated with distance between groups of populations of *S. serrata* ($r = 0.161$; $p = 0.29$; Figure 6.4D). This implies that there was no relationship between gene flow and geographic distance between populations in *S. serrata*.

The dendrogram generated by an UPGMA from Nei's genetic distances showed no grouping of *S. serrata* populations by coastal region (Figure 6.6). Within the dendrogram, the two main groups of populations contained random assortments of populations from each coastal region. This reinforces the previous F_{ST} and Nm results, which showed fairly high, random genetic differentiation among populations.

6.3.2.1 Small-scale analysis of *S. serrata* populations

Only three (FUM, MDH, NP) of the four polymorphic loci for *S. serrata* were found to show variation in the small-scale study area between the Cape and Transkei coastal regions. G6PDH was found to be monomorphic in these populations as was the case along the Transkei coast (Appendix V). Within the populations sampled on a small scale only NP showed no variation in population I (Table 6.6). The proportions of polymorphic loci appeared greater in populations on a small scale than those scattered over long distances along the coast (Table 6.6 and Appendix V).

Mean gene diversity (H) ranged from 0.000 to 0.501 within populations I to V (Table 6.7). This is a greater range than that found in *S. serrata* populations separated by larger distances (Appendix VI).

Inbreeding (F_{IS} = 0.332; Table 6.8) occurred in these populations at very similar levels to that found in the populations of *S. serrata* examined on a large scale (F_{IS} = 0.323; Table 6.4). As previously stated, this suggests that a substantial amount of inbreeding occurred within this species. Meanwhile, genetic differentiation among populations on a small scale was lower (F_{ST} = 0.08; Table 6.8) than that of populations spread along the entire coast (F_{ST} = 0.395; Table 6.4). This suggests that a greater degree of genetic interaction occurred among populations of *S. serrata* on a small scale. This indicates that there is less genetic differentiation among populations of *S. serrata* on a small scale as compared to the large scale.

Pairwise F_{ST} values for the small-scale populations of *S. serrata* ranged from 0.000 to 0.103 (Table 6.9). This is similar to the range of F_{ST} pairwise values found on a large scale (Table 6.5).

The pairwise Nm values showed that gene flow occurred between each and every pair of populations, as all the Nm values were greater than 1 (Table 6.9). These Nm values were found not to be significantly correlated with geographic distance between populations (r = 0.461; p = 0.180; Figure 6.7).

The dendrogram generated by an UPGMA from Nei's genetic distances showed that the small-scale populations formed two groups (Figure 6.8). Populations II and V

were very similar to one another, while populations I, III and IV formed a separate group. The populations did not link most closely to their closest geographic neighbour. This suggests that there is substantial gene flow may occur over the 90km distance separating these populations. This is supported by the relatively high gene flow values (Table 6.9).

While large-scale (up to 1400km) genetic differentiation and gene flow failed to exhibit a definite pattern in *S. serrata*, over a small distance of 90km genetic differentiation was lower and gene flow certainly occurred among all populations. This suggests that there is genetic cohesion among populations of this species on a small scale (<100km).

6.3.3 Comparison between species

The proportions of polymorphic loci within *S. serrata* were significantly lower than those within populations of *S. capensis* at the same sites (Wilcoxon matched pairs test; $T = 22$; $Z = 3.528$; $p<0.001$).

Mean gene diversity values ranged from 0.033 to 0.300 in *S. capensis* and from 0.021 to 0.275 (Figure 6.3C and D) in *S. serrata*. A Wilcoxon match paired test showed a significant difference between these mean H values, with those of *S. serrata* being significantly lower than those of *S. capensis* ($T = 61$; $Z = 2.127$; $p < 0.033$).

Interestingly, the overall F_{IS} values for both species were high and similar to one another (*S. capensis*: $F_{IS} = 0.582$; *S. serrata*: $F_{IS} = 0.543$), suggesting that inbreeding occurred in both species. However, the overall F_{ST} values were different between the

two species. When the entire sample range is considered, F_{ST} values were higher in *S. serrata* (0.395) than in *S. capensis* (0.251) (Tables 6.2 and 6.4). Surprisingly, both species showed similar levels of genetic differentiation among populations along the Transkei coast (*S. serrata*: $F_{ST} = 0.298 \pm 0.11$; *S. capensis*: $F_{ST} = 0.224 \pm 0.099$). While along the Cape coast, genetic differentiation among populations of each species was vastly higher in *S. serrata* (*S. capensis*: $F_{ST} = 0.228$; *S. serrata*: $F_{ST} = 0.486$).

A clear link between distance between groups of populations and pairwise F_{ST} values was found in *S. capensis*. The lowest F_{ST} values occurred between neighbouring populations, e.g. $F_{ST} = 0.005$ between groups E and F (Table 6.3). While, groups at opposite ends of the sampling range, namely groups A and F showed higher genetic differentiation ($F_{ST} = 0.126$; Table 6.3). In contrast, the pairwise F_{ST} values of *S. serrata* appeared erratic and there was no clear relationship between genetic differentiation and geographical distance between groups (e.g. groups A and F some 1400km apart showed a low F_{ST} value of 0.016; Table 6.5).

Significant gene flow (calculated as Nm) was found to occur in both *S. capensis* and *S. serrata* as the majority of Nm values were greater than one (Slatkin, 1987). However, gene flow occurred on a much greater level in *S. capensis*. Gene flow was certainly correlated to distance between groups in *S. capensis*, while there was no such correlation in *S. serrata*, where gene flow values appeared chaotic at best (Figure 6.4B and D).

Dendrograms generated by an UPGMA based on Nei's genetic distance values once again illustrated the contrast in genetic relatedness of populations within each species.

Within *S. capensis*, most populations grouped together according to their geographic location (Figure 6.5). Within *S. serrata*, a random assortment of populations was found within two major groups (Figure 6.6).

6.4 DISCUSSION

Siphonaria capensis conformed to predictions that its pelagic larvae disperse over long distances (100s km), allowing substantial gene flow among populations. In *S. serrata* populations, there was no obvious pattern of genetic differentiation or gene flow on a large scale. This confirmed predictions that populations of this species are not strongly linked by gene flow. However, populations of *S. serrata* were linked genetically on smaller scales (10s km).

Siphonaria capensis appeared to have slightly greater levels of genetic differentiation than most of the examples of the planktonic developers given in Table 6.10. Genetic differentiation in *S. capensis* was similar to that of the South African limpet *Patella barbara*, and fell within the range calculated for the scleractinian coral, *Mycedium elephantotus* by Yu *et al.* in 1999 (Table 6.10). *Siphonaria serrata* showed no pattern of gene flow and the levels observed were similar to those for the snail *Bembicium vittatum* (Table 6.10). Generally, both *Siphonaria* species conformed approximately to the genetic differentiation and gene flow values of other organisms with similar forms of reproductive development (Table 6.10).

Focusing more closely on each species, high levels of genetic variation were found within populations of *S. capensis*. This was illustrated by the proportions of

polymorphic loci and the H values (Figure 6.3A and B). This suggests that, as expected, larval dispersal caused populations to be composed of individuals originating from several different parent populations.

Genetic differentiation among *S. capensis* populations was relatively high for a species with planktonic larval dispersal, however, in comparison to *S. serrata*, genetic differentiation was low in this species (F_{ST} values; Table 6.3). Long range gene flow was found to occur in *S. capensis* (up to 1600km; Figure 6.4B). These results suggest that larval dispersal in this species is effective and allows substantial gene flow. The geographic range of gene flow in *S. capensis* is similar to that found for the periwinkle snail, *Nodilittorina africana knysnaensis* (Grant and Lang, 1991), the brown mussel, *Perna perna* (Grant *et al.*, 1992) and the limpet, *Patella barbara* (Ridgway *et al.*, 1998), all studied along the South African coast.

Examining populations of the direct developer *S. serrata*, genetic variation within populations was found to be relatively low. This was shown by the low proportions of polymorphic loci and low gene diversity values (Figure 6.3C and D). This was expected, due to the reduced dispersal potential of this species as the effective population size is reduced and less genetic variation is expected (Varvio-Aho, 1983 cited by Russo *et al.*, 1994). In such populations, there are fewer "incoming" individuals, which keeps populations isolated, so that genetic drift can take place independently within each population. Ultimately, this leads to the fixation of loci and lower within-population genetic variation, as observed.

Siphonaria serrata was found to have relatively high among-population differentiation, illustrated by high F_{ST} values (Table 6.5). No obvious patterns of gene

flow were found in *S. serrata*. However, all gene flow values were greater than 1, indicating that at least some gene flow occurred. So, although gene flow on a large scale was erratic, it was sufficient to maintain genetic connectedness of populations of this species and so prevent eventual speciation through local adaptation.

Genetic differentiation within the direct developer, *S. serrata* appeared to be independent of scale. Inbreeding (F_{IS}) levels were similar on both a small and large scale. The similar pairwise F_{ST} values and lack of correlation between gene flow and geographic distance between populations suggest that genetic cohesion among populations on a small scale is not significantly greater than on a large scale. Interestingly, the populations in both studies formed two groups, which linked at approximately the same genetic distance (Figures 6.6 and 6.7). The most striking difference between the large and small-scale studies lies in the mean H values. Gene diversity (H) showed a greater range in the small-scale study. This is probably due to chance, rather than a result of decreased genetic interaction.

The patterns of genetic variation seen in *S. capensis* and *S. serrata* could be due to:

1. high gene flow from extensive dispersal (this enhances within-population variation and decreases among-population differentiation)
2. genetic drift (this decreases within-population genetic variation and increases among-population differentiation)
3. balance of selection acting on all loci (again, this decreases within-population genetic variation and increases among-population differentiation) (Hellberg, 1996)
4. rapid expansion of founder populations (this decreases within-population variation and increases among-population differentiation) (Williams and Benzie, 1996)

Several authors consider that, although planktonic dispersal occurs, genetic similarity among populations is due to selection acting on the same alleles in geographically distinct populations (Marcus, 1977; Slatkin, 1985; 1987; Johnson and Black, 1994; Williams and Benzie, 1996). Nevertheless, correlations between a high capacity for dispersal and high rates of gene flow exist for several species with planktonic larvae (Liu *et al.*, 1991; Hunt, 1993; Williams and Benzie, 1993; Hurst and Skibinski, 1995; Murray-Jones and Ayre, 1997; Gallardo *et al.*, 1998 and examples in Table 6.10). *Siphonaria capensis* shows a strong correlation between its high gene flow levels and distance between groups of populations and distance of the Agulhas Current from the shore (see below), strongly suggesting that gene flow is linked to long range dispersal of pelagic larvae. The high gene diversity values (Figure 6.3B; Appendix III) found within populations of *S. capensis* may suggest that selection does not play a strong role as it would result in low within-population variation. In addition, the loci examined were found to be in Hardy-Weinberg equilibrium, suggesting that selection is not a cause of genetic similarity among populations.

Dispersal of marine invertebrates, such as *S. capensis,* is likely to be influenced by the hydrography of a region (Shepherd *et al.*, 1992; Bertness and Gaines, 1993; Kojima *et al.*, 1997; Brierley and Brandon, 1999; Hilbish *et al.*, 2002). Ocean currents often determine the distance and direction travelled by larvae (Scheltema, 1971; 1975; Olson, 1985; Todd *et al.*, 1988; Hunt, 1993; Hellberg, 1996; Murray-Jones and Ayre, 1997; Yu *et al.*, 1999). Studies on the pulmonate gastropod *Melampus bidentatus* (Schaeffer *et al.*, 1985) and on a coral, *Mycedium elephantotus*, inhabiting southern Taiwan (Yu *et al.*, 1999) revealed a direct correlation between strength and direction

of ocean currents and level of gene flow. Genetic differentiation of the Japanese turban shell, *Turbo (Batillus) cornutus*, was also found to be strongly influenced by warm currents among the Japanese islands (Kojima *et al.*, 1997). However, as is usually the case in biology, there are counter examples and gene flow may not always parallel the major ocean surface currents. A study on giant clams (*Tridacna gigas* and *T. maxima*) in the Indo-Pacific region found that, while gene flow was high within coastal regions, it appeared to be perpendicular to the direction of the ocean currents between coastal regions (Benzie and Williams, 1995 and 1997).

The major oceanic currents around the coast of South Africa are the Agulhas and Benguela systems (see Figure 2.2, Chapter 2). The Agulhas Current flows very close inshore (5 to 10km) along the Transkei coast of South Africa (Duncombe Rae, 1991). This has been found to be responsible for transporting various species of fish larvae in a southerly direction along the Transkei coast (Beckley, 1994). From East London southwards, the continental shelf widens rapidly, and in so doing it pushes the Agulhas Current out to sea. As the current reaches Cape Agulhas, it is approximately 270km offshore and its influence on the shore becomes minimal (Harris, 1978; Schumann *et al.*, 1982; 1988). In the western region of the Cape coast, the Agulhas Current approaches the Benguela system. The Benguela Current flows northwards from the Cape of Good Hope along the west coast of South Africa (Duncombe Rae, 1991). Cape Agulhas is considered to be the most appropriate boundary between the Agulhas and Benguela Currents (Brown, 1992; Largier *et al.*, 1992). The boundary between the two systems was expected to limit larval dispersal. The inshore fast flowing Agulhas Current and its inshore countercurrents along the Transkei coast almost certainly assist larval dispersal. Evidence that the Agulhas Current assists

larval dispersal of *S. capensis* along the Transkei coast lies in the high Nm values (Table 6.3) and in the fact that the results for Nei's genetic distances linked Mossel Bay (15) and Stilbaai (14) populations more closely to those along the Transkei coast than to their geographic neighbours along the Cape coast (Figure 6.5). The high dynamic variability of the inshore currents shoreward of the Agulhas Current along the Cape coast may limit dispersal. This is probably the cause of the lower levels of gene flow found among groups A, B and C in *S. capensis* (Table 6.3). Gene flow across the proposed boundary at Cape Agulhas was low, but certainly occurred (Nm between groups A and B = 3.46; Table 6.3). In fact, Largier *et al.* (1992) found evidence of warm water frequently moving north-westerly up the west Agulhas bank, around Cape Point and up the west coast, sometimes as far as St Helena Bay (see Figure 2.2, Chapter 2). This frontal jet stream was found to transport anchovy eggs and larvae from Cape Agulhas to the west coast nursery grounds (Largier *et al.*, 1992) and probably aids larval dispersal in *S. capensis*. Thus larval dispersal and hence gene flow of *S. capensis* are influenced by ocean currents along the entire coast of South Africa, as is also shown by the multiple regression between gene flow and distance between groups of populations and distance of the Agulhas Current from the coast ($r^2 = 0.73$; $p < 0.05$).

Considering possible causes of the genetic variation found in *S. serrata*, it is clear that substantial dispersal does not occur in this species. However, it is thought that species with limited dispersal potential may disperse through one or both of the following mechanisms: drifting (passive transport) of whole post-larval organisms in the ambient currents (Scheltema, 1975; Bingham and Young, 1991; Martel and Chia, 1991; Tatarenkov and Johannesson, 1994; Grant and da Silva-Tatley, 1997;

Ruckelshaus, 1998) or rafting of egg masses (Hummel *et al.*, 1997) or adults on objects such as algae, logs, seagrasses, pumice (Worcester, 1994) or even attached to birds legs, or in ships ballast's etc. (Johannesson and Warmoes, 1990). There is controversy on this topic (which is relatively unstudied) but drifting and/or rafting have been shown to occur in a small prosobranch gastropod *Barleeia sp.*; in brooding bivalves, *Musculus sp.*, *Lasaea sp.*, and *Transennella tantilla*; several unidentified juvenile limpets (O' Foighil, 1989; Martel and Chia, 1991); and in eelgrass (*Botrylloides sp*) colonies (Worcester, 1994). Indeed, many organisms have been found rafting on eelgrass (Worcester, 1994). Johannesson and Warmoes (1990) suggested that prehatching of direct developing embryos may occur and so aid in the dispersal of such a species. The chances of any of these dispersal events occurring and the juveniles surviving are slim. Such dispersal was certainly not found to contribute significantly to gene flow in the whelk, *Nucella lamellosa* (Grant and Utter, 1987). Rafting of *S. serrata* individuals is possible, though it is probably an infrequent event. Much of the South African coastline is composed of exposed shores (Branch and Branch, 1992), and this will further limit dispersal through rafting (Todd *et al.*, 1998). Some rafting by *S. serrata* may occur and explain the gene flow among populations close to one another (populations I to V). The relatively high level of gene flow along the Transkei coast (Table 6.5) may be due rafting assisted by the close-to-shore Agulhas Current. Rare though these "alternate" dispersal events may be, they are thought to be important in allowing genetic cohesiveness among populations (C D Todd, *pers comm*). A single individual dispersing between populations in a single generation is sufficient to prevent strong genetic differentiation among populations (Slatkin, 1987) and so could be part of the cause of the results seen in *S. serrata*.

Alternatively, selection may favour different alleles in different populations, in which case differentiation will reflect genetic adaptation to local conditions (Slatkin, 1987), so resulting in high genetic variation among populations as seen in *S. serrata*. However, evidence of selection acting on allozyme loci is debated. Johannesson and Tatarenkov (1997) found that allozyme loci were under selection, though others have been uncertain if selection has any real effect on allozymic loci (Skelton, 1983; Karl and Avise, 1992; Todd *et al.*, 1994). Todd *et al.* (1988 and 1994) and Watts *et al.* (1990) suggest that the only firm conclusion that can be drawn is that, if selection acts on allozymic loci, its effects are small and differentiation among populations is more likely to be caused by founder effects, bottlenecks or local extinctions. Selection is also expected to cause deviation from Hardy-Weinberg equilibrium (Marcus, 1977), which was not found for *S. serrata*. While the loci examined were selectively neutral, unexamined loci may of course be under the influence of selection.

Both *Siphonaria* species have similar ecological requirements and so it can be argued that the different levels of genetic variation could be due to stochastic phenomena (Russo *et al.*, 1994). One such stochastic process is genetic drift. This is defined as an unpredictable change in gene frequency in a finite population and, like gene flow, is expected to have the same average effect on all nuclear genes (Slatkin, 1987; Skelton, 1993). Genetic drift and rapid expansion of populations can be combined and together with selection can be more accurately described as the "shifting balance theory" (Wright, 1978). This theory assumes that many species comprise small, partially isolated populations. Dispersal between these established populations, as well as extinction and recolonisation events, is assumed to occur and allow for the spreading of new well-adapted gene combinations (Slatkin, 1985 and 1987; Hartl and Clark,

1989). There is debate as to the results of these processes. Slatkin (1987) suggests that extinction and recolonisation are important sources of gene flow and would in fact lower genetic differentiation among populations. Wade and McCauley (1988), on the other hand, suggest that these processes will result in higher genetic differentiation. Through a series of complex calculations, Wade and McCauley (1988) showed that in fact the answer depends on the number of individuals establishing new colonies (propagules) relative to the number of individuals moving among populations (migrants). There is little, if any, evidence to suggest that extinction and recolonisation commonly enhance gene flow, instead, these processes were found to promote local genetic population differentiation (Wade and McCauley, 1988). Genetic models such as this, are particularly difficult to construct and remain largely untested (Slatkin, 1987). However, studies on eelgrass (*Zostera marina*) along the Washington state coast (Ruckelshaus, 1998), and thermal vent alvinellid polychaete populations (Jollivet *et al.*, 1995) showed high genetic variation as a result of successive extinction and recolonisation events. For these processes to occur, populations must be fairly isolated, and migration/dispersal between neighbouring populations must be great enough for adaptive gene combinations to spread (Hartl and Clark, 1989). Extinction and recolonisation events may be responsible for the absence of a pattern in genetic differentiation among *S. serrata* populations, especially in view of the fact that some gene flow occurred among populations. Nevertheless, alternate dispersal strategies are a more convincing explanation for the high levels of genetic variation found in *S. serrata*, especially along the Transkei coast where gene flow was surprisingly high. Finally, the apparent lack of a systematic pattern of genetic differentiation and gene flow among populations of *S. serrata* may simply be due to the species being composed of several independent populations which have little

interaction with one another.

Lastly, through his extensive review on gene flow, Slatkin (1985) drew some tentative conclusions: gene flow varies greatly among species; different levels of gene flow may not necessarily reflect morphological appearances; gene flow may be sporadic in species where dispersal over short distances is frequent but long distance dispersal only occurs often enough to maintain wide spread genetic homogeneity; and indirect methods are useful for estimating average levels of gene flow. This study supports some of these ideas. Gene flow and genetic structure of populations did vary greatly between *S. capensis* and *S. serrata*. Gene flow in *S. serrata*, the direct developer, appeared sporadic, but was certainly sufficient to maintain wide spread genetic links among populations and was relatively high over short distances. Finally, allozyme analysis is clearly useful for estimating rates of gene flow in these marine limpets.

6.5 CONCLUSION

To conclude, it was found that *S. capensis* did indeed conform to the original hypothesis. This species exhibited high within-population variation and low among-population differentiation. This is a result of the high levels of larval dispersal, which in turn produced high gene flow. Ocean currents influenced larval dispersal of *S. capensis*. Genetic variation within populations of *S. serrata* was found to be low. The lack of an obvious pattern of among-population differentiation and relatively low levels of gene flow found in the direct developer, *S. serrata* were expected. However, gene flow in *S. serrata* was sufficient to maintain genetic cohesion of populations and this may have been achieved through alternate dispersal strategies.

Table 6.1: Loci examined, electrophoretic conditions and number of alleles observed at each locus

Enzyme	Abbreviation	EC Number	Buffers	pH	Number of alleles	Species
Aspartate aminotransferase	AAT	2.6.1.1	TG (1:5)	8.6	2	*S.capensis*
Acid phosphatase	ACP	3.1.3.2	TG (1:5)	8.6	2	*S.capensis*
Aconitase	AD	4.2.1.3	TG (1:5)	8.6	1	
Adenosine deaminase	ADA	3.5.4.4	TG (1:5)	8.6	1	
Aldehyde oxidase	AO	1.2.3.1	TG (1:5)	8.6	1	
Fructose-biphosphate aldolase	FBALD	4.1.2.1.3	TG (1:5)	8.6	1	
Fumerase	FUM	4.2.1.2	TG (1:3)	8.6	2	*S. serrata*
Guanine deaminase	GDA	3.5.4.3	TG (1:5)	8.6	1	
Glycerophosphate dehydrogenase	GPDH	4.2.1.2	CAEA (1:4)	7.2	1	
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	TG (1:5)	8.6	1	
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	TG (1:5)	8.6	2	*S. serrata*
Isocitrate dehydrogenase	IDH	1.1.1.42	TG (1:5)	8.6	1	
Leucine aminopeptidase	LAP	3.4.11.1	CAEA (1:4)	7.2	1	
Malate dehydrogenase	MDH	1.1.1.37	TG (1:5)	8.6	2	*S. capensis* and *S. serrata*
Malic enzyme	ME	1.1.1.40	TG (1:5)	8.6	1	
Mannose-6-phosphate isomerase	MPI	5.3.1.8	TG (1:5)	8.6	2	*S.capensis*
Nucleoside phosphorylase	NP	2.4.2.1	TG (1:5)	8.6	2	*S. serrata*
Phosphoglucoisomerase	PGI	5.3.1.9	TG (1:5)	8.6	1	
Phosphoglucomutase	PGM	5.4.2.2	CAEA (1:4)	7.2	2	*S.capensis*
6-phosphogluconate dehydrogenase	6PGDH	1.1.1.44	TG (1:5)	8.6	1	

Table 6.2: Wright's F statistics for all populations of *S. capensis* reflected by five polymorphic loci, corresponding standard errors are in brackets.

Locus	F_{IS}	F_{IT}	F_{ST}
AAT	0.446 (±0.108)	0.510 (±0.067)	0.115 (±0.050)
ACP	0.434 (±0.000)	0.523 (±0.177)	0.157 (±0.033)
MDH	0.093 (±0.007)	0.181 (±0.004)	0.097 (±0.003)
MPI	0.622 (±0.089)	0.709 (±0.073)	0.230 (±0.013)
PGM	0.662 (±0.032)	0.819 (±0.005)	0.465 (±0.066)
MEAN	0.466 (±0.305)	0.601 (±0.264)	0.251 (±0.082)

Table 6.3: Pairwise matrix of Wright's F_{ST} index (above diagonal) and estimated pairwise migration rates, Nm (below diagonal) for *S. capensis* populations. Group symbols A to F correspond to groupings of single populations as shown in Figure 6.2.

	A	B	C	D	E	F
A	----------	0.0674	0.0617	0.0898	0.1224	0.1263
B	3.4604	----------	0.0914	0.0837	0.0627	0.0820
C	3.7995	2.4851	----------	0.0329	0.0421	0.0430
D	2.5338	2.7385	7.3444	----------	0.0162	0.0264
E	1.7918	3.7361	5.6941	15.2173	----------	0.0054
F	1.7292	2.7971	5.5646	9.2350	46.1090	----------

Table 6.4: Wright's F statistics for all populations of *S. serrata* reflected by four polymorphic loci, corresponding standard errors are in brackets.

Locus	F_{IS}	F_{IT}	F_{ST}
FUM	0.663 (±0.115)	0.858 (±0.047)	0.578 (±0.004)
G6PDH	0.604 (±0.020)	0.663 (±0.024)	0.149 (±0.006)
MDH	-0.046 (±0.106)	0.219 (±0.146)	0.253 (±0.065)
NP	0.672 (±0.000)	0.722 (±0.002)	0.153 (±0.000)
MEAN	0.323 (±0.533)	0.590 (±0.413)	0.395 (±0.288)

Table 6.5: Pairwise matrix of Wright's F_{ST} index (above diagonal) and estimated pairwise migration rates, Nm (below diagonal) for *S. serrata* populations. Group symbols A to F correspond to groupings of single populations as shown in Figure 6.2.

	A	B	C	D	E	F
A	----------	0.0082	0.1247	0.0973	0.0714	0.0164
B	30.3700	----------	0.1498	0.1138	0.1106	0.0398
C	1.7550	1.4192	----------	0.0484	0.0456	0.0925
D	2.3197	1.9473	4.9188	----------	0.0206	0.0489
E	3.2491	2.0112	5.2304	11.9144	----------	0.0296
F	15.0160	6.0245	2.4515	4.8655	8.2014	----------

Table 6.6: Allele frequency of polymorphic loci of *S. serrata* populations over a small scale. (p(A or B) = proportions of allele A or B; n = sample size)

Enzyme	Population I	II	III	IV	V
FUM					
p(A)	0.8462	0.9625	0.9000	0.9625	0.9625
p(B)	0.1538	0.0375	0.1000	0.0375	0.0375
n	40	40	40	40	40
MDH					
p(A)	0.8846	0.6250	0.8205	0.8864	0.5526
p(B)	0.1154	0.3750	0.1795	0.1154	0.4474
n	40	40	40	40	40
NP					
p(A)	1.0000	0.9750	0.9125	0.9875	0.9625
p(B)	0.0000	0.0250	0.0875	0.0125	0.0375
n	40	40	40	40	40

Table 6.7: Values of gene diversity (H) for polymorphic loci of *S. serrata* populations on a small scale (Grant, 1994). G is expected gene diversity.

Enzyme	Population I	II	III	IV	V
FUM					
H	0.2637	0.0731	0.1823	0.0731	0.0731
G	0.4416	0.0379	0.1022	0.0006	0.0379
MDH					
H	0.2068	0.4747	0.2984	0.2068	0.5011
G	0.0000	0.4269	0.0918	0.0746	0.3038
NP					
H	0.0000	0.0494	0.1617	0.0250	0.0731
G	n/a	0.0653	0.0034	0.0000	0.0006
Mean					
H	0.0833	0.1063	0.1075	0.0571	0.0908
G	0.1176	0.1493	0.1606	0.0762	0.1618

Table 6.8: Wright's F statistics for populations of *S. serrata* on a small scale, reflected by three polymorphic loci, corresponding standard errors are in brackets.

Locus	F_{IS}	F_{IT}	F_{ST}
FUM	0.510 (±0.031)	0.520 (±0.027)	0.022 (±0.004)
MDH	0.273 (±0.164)	0.353 (±0.194)	0.110 (±0.059)
NP	0.275 (±0.174)	0.290 (±0.188)	0.020 (±0.027)
Mean	0.332 (±0.141)	0.386 (±0.010)	0.080 (±0.053)

Table 6.9: Pairwise F_{ST} (above the diagonal) and Nm (below the diagonal) values of *S. serrata* populations on a small scale.

	I	II	III	IV	V
I	--------	0.070	0.025	0.019	0.103
II	3.035	--------	0.036	0.051	0.000
III	9.764	6.778	--------	0.019	0.052
IV	12.632	4.650	12.913	--------	0.076
V	2.171	infinite	4.537	3.058	--------

Table 6.10: Gene flow in various marine invertebrate species

Species	Common name	Development	Region	Fst	Nm[1]	Geographic range of calculated gene flow	Reference
Littorina striata	periwinkle	planktonic	Macronesian archipelagos	0.044	5.40	10 - 2000 km	De Wolf *et al.*, 1998a
Littorina littorea	periwinkle	planktonic	Sweden	0.021 (Gst)		5m to 288km	Janson, 1987
Littorina littorea	periwinkle	planktonic	Sweden	0.030 (Gst)		throughout Europe	Johannesson, 1992
Melarhaphe (L.) neritoides	periwinkle	planktonic	Sweden	0.035 (Gst)		throughout Europe	Johannesson, 1992
Littorina cingulata	periwinkle	planktonic	Western Australia	0.031	*7.80*	500km	Johnson and Black, 1998
Littorina striata	periwinkle	planktonic	Ilheu	0.013-0.022	22.60	few metres	De Wolf *et al.*, 1998c
Drupella cornus	snail	planktonic	Western Australia	0.044	*5.43*	80m	Johnson *et al.*, 1993
				0.028	*9.00*	1.6km	
				0.014	*17.61*	119km	
				0.007	35.46	180km	
Stramonita haemostoma	snail	planktonic	SE coast of USA	-0.001	*249.75*	<50km	Liu *et al.*, 1991
				0.000		<500km	
				-0.001	*249.75*	<1500km	
Patella vulgata	limpet	planktonic	British Isles	0.016-0.027	*8.70-15.40*	within regions	Hurst and Skibinski, 1995
				0.028	9.00	among regions	
Patella barbara	limpet	planktonic	southern Africa	0.124	*1.77*	~1500km	Ridgway *et al.*, 1999
Siphonaria kurracheensis	limpet	planktonic	Western Australia	0.010	*25.00*	400km	
				0.009	*27.50*	70km	Johnson *et al.*, 2001
Perna perna	mussel	planktonic	southern Africa	0.030	*7.80*	1700km	Grant *et al.*, 1992
Donax deltoides	bivalve	planktonic	east Australia	0.009	27.50	1200km	Murray-Jones and Ayre, 1997
Concholepas concholepas	bivalve	planktonic	southern Chile	0.021	~11	~1000km	Gallardo and Carrasco, 1996
Mytilus galloprovincialis	mussel	planktonic	Mediterranean	0.029 (Gst)		~4000km	Quesada *et al.*, 1995
Mytilus californianus	mussel	planktonic	NW America	0.021	11.00	1000km	Levinton and Suchanek, 1978
Mytilus edulis	mussel	planktonic	NW America	0.004-0.160	*1.31-62.3*	~1000km	Levinton and Suchanek, 1978
				0.005	*49.75*	~100000km	
Venus antiqua	clam	planktonic	southern Chile	0.007	*35.46*	~300km	Gallardo *et al.*, 1998
				0.143	*1.50*	~1800km	

Table 6.10 (continued)

Species	Common name	Development	Region	F_{ST}	Nm[1]	Geographic range of calculated gene flow	Reference
Tridacna maxima	giant clam	planktonic	west Indo-Pacific, incl. GBR	0.000-0.044	>83.00	within reef groups	Benzie and Williams, 1997
				0.070	2.00-5.00	within regions	
				0.156	<1.40	among regions	
Strombus gigas	queen conch	planktonic	Caribbean	0.010-0.220	6.80 -8.70	~1500km	Mitton *et al.*, 1989
Linckia laevigata	starfish	planktonic	Great Barrier Reef, Australia	0.001	249.80	1000km	Williams and Benzie, 1993
			east-Indian west-Pacific	0.002	*124.75*	>6500km	Williams and Benzie, 1996
Echinometra mathaei	sea urchin	planktonic	Western Australia	0.013	*19.44*	1300km	Watts *et al.*, 1990
Echinothrix diadema	sea urchin	planktonic	Eastern Pacific Barrier	0.064	3.66	~6000km	Lessios *et al.*, 1998
Holothuria scabra	sea cucumber	planktonic	Great Barrier Reef, Australia	0.02	9.5	1300km	Uthicke and Benzie, 2001
Holothuria nobilis	sea cucumber	planktonic	Great Barrier Reef, Australia	0.002	*~125*	1300km	Uthicke and Benzie, 2000
*Adalaria proxima**	nudibranch	planktonic	British Isles	0.306	*0.57*	26km	Todd *et al.*, 1998
Goniodoris nodosa	nudibranch	planktonic	British Isles	-0.003	*83.08*	~1600km	Todd *et al.*, 1998
Bunodosoma caissarum	sea anemone	planktonic	Brazil	0.042	*5.70*	1150km	Russo *et al.*, 1994
Synalpheus pectiniger	sponge-dwelling shrimps	planktonic	Caribbean	0.002-0.058	125.00	within regions	Duffy, 1993
				0.143	1.50	among regions	
Nephrops norvegicus	lobster	planktonic	Mediterranean	0.122	1.80	~2000km	Maltagliati *et al.*, 1998
Paracyathus stearnsii	solitary coral	planktonic	California	0.004	~60	~1000km	Hellberg, 1996
Mycedium elephantotus	coral	planktonic	Taiwan	0.010-0.022	11.1- 24.8	within regions	Yu *et al.*, 1999
				0.032-0.218	0.90-7.56	among regions	

Table 6.10 (continued):

Species	Common name	Development	Region	F_{ST}	Nm[1]	Geographic range of calculated gene flow	Reference
Littorina saxatilis complex	periwinkle	direct	British Isles	0.142	*1.51*	around British coast	Knight and Ward, 1991
Littorina saxatilis	periwinkle	direct	Sweden	0.078 (Gst)		30m to 288km	Janson, 1987
*Littorina saxatilis**	periwinkle	direct	Sweden	0.073	*3.17*	1km	Janson and Ward, 1984
				0.053	*4.47*	50km	
Bembicium vittatum	snail	direct	Western Australia	0.163	*1.28*	all populations	Johnson and Black, 1991
				0.277	*0.16*	lake populations	
				0.138	*1.56*	protected lagoon	
				0.022	*11.10*	exposed shore	
*Bullia digitalis**	whelk	direct	southern Africa	0.013	19.00	~1000km	Grant and da Silva-Tatley, 1997
Nucella lamellosa	whelk	direct	north west America	0.286	*0.62*	~500km	Grant and Utter, 1987
Actinia bermudensis	sea anemone	direct	Brazil	0.262	*0.70*	1150km	Russo *et al.*, 1994
*Pocillopora damicornis**	brooding coral	direct	G B R, Australia	0.084	2.60-6.90	within reefs	Ayre *et al.*, 1997
					31.00	among reefs >1000km	
Balanophyllia elegans	solitary coral	direct	California	0.200	~1	~1000km	Hellberg, 1996
Synalpheus brooksi	sponge-dwelling shrimps	direct	Caribbean	0.064-0.214	0.92-3.64	within regions	Duffy, 1993
				0.538	0.21	among regions	
Pareledone turqueti	octopus	direct	Antarctic	0.740	*0.09*	150km	Allcock *et al.*, 1997

* denotes contradiction of the theory that planktonic development results in high gene flow or direct development results in high genetic differentiation among populations

[1]Nm value in italics calculated from Fst by current author

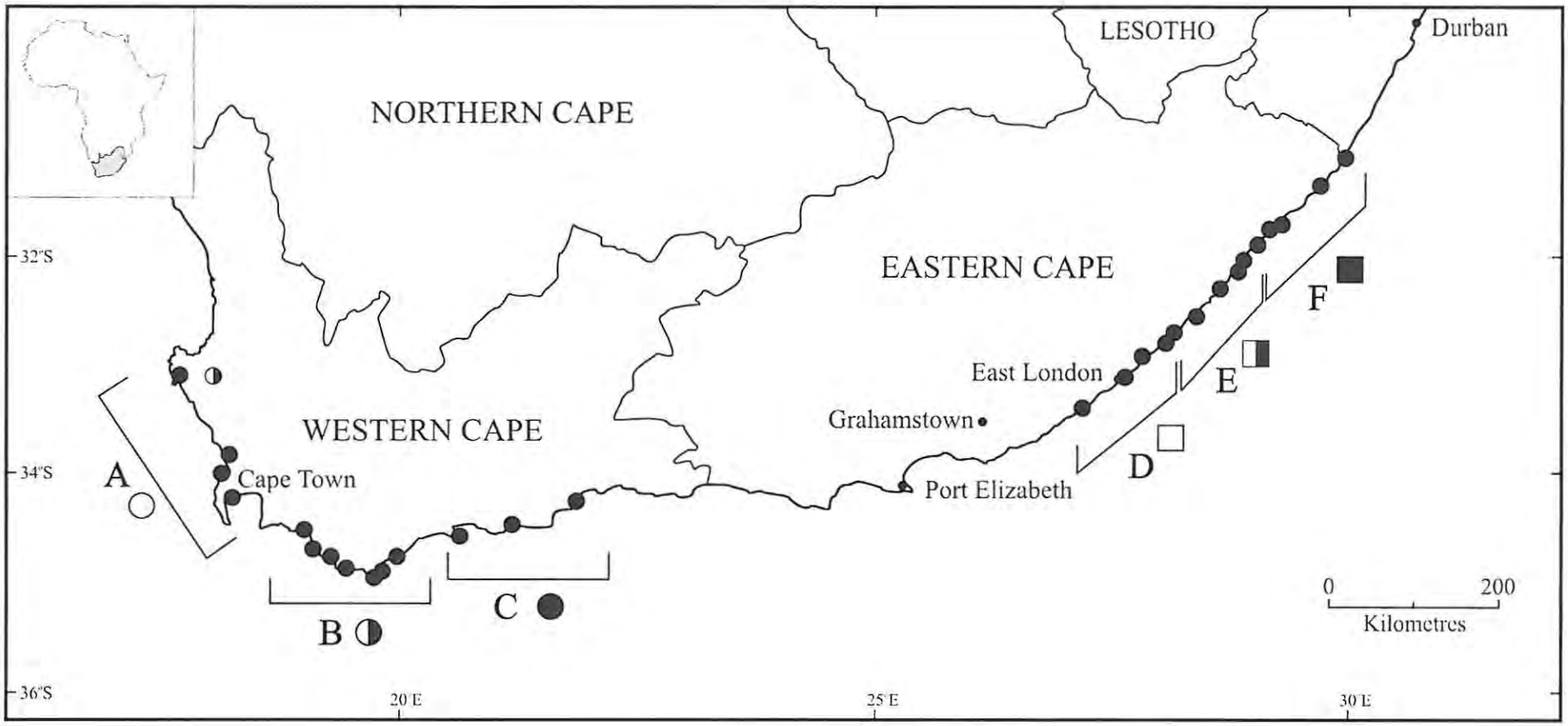


Figure 6.1: Map showing grouping of populations for pairwise comparisons of F_{ST} and Nm values in both species. Sites are labelled according to whether one or both species were collected, i.e. ○ *Siphonaria capensis* only; ● *S. serrata* only; ◑ both *S. capensis* and *S. serrata*. Populations were grouped as follows: Group A = sites 1, 2, 3,5; Group B = sites 6, 7, 8, 9, 10, 11, 12; Group C = sites 13, 14, 15; Group D = 16, 17, 18, 19; Group E = 20; 21, 22, 23, 24, 25; Group F = 26, 27, 28, 29. Sites I to V were used in the small scale study of *S. serrata* only.

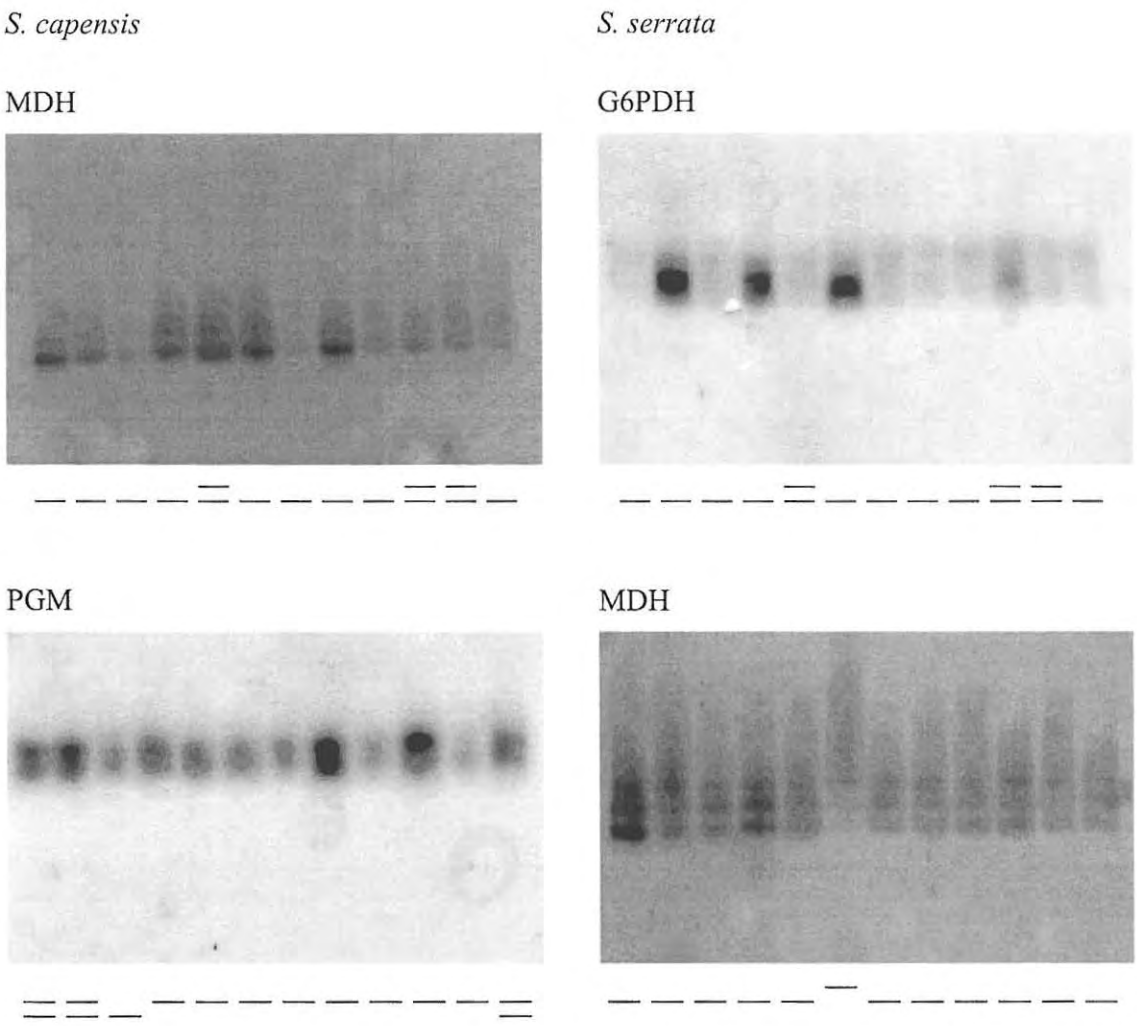
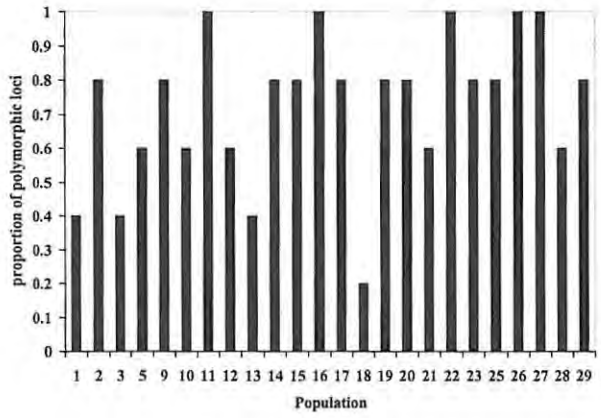


Figure 6.2: Examples of allozymes run on cellulose acetate gels for *S. capensis* and *S. serrata*.

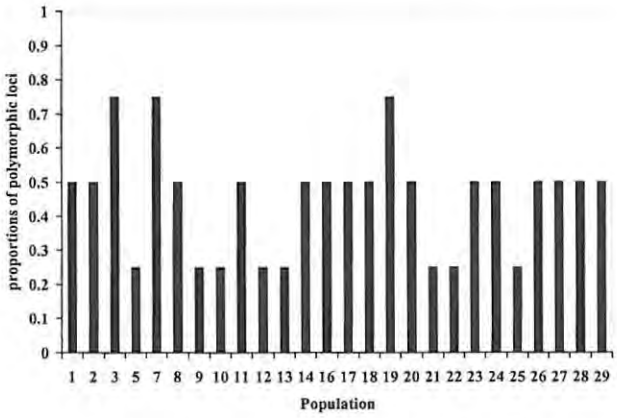
S.capensis

A: proportions of polymorphic loci

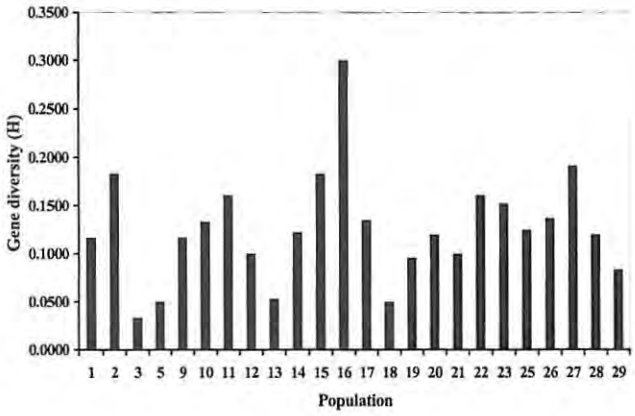


S. serrata

C: proportions of polymorphic loci



B: gene diversity (H)



D: gene diversity (H)

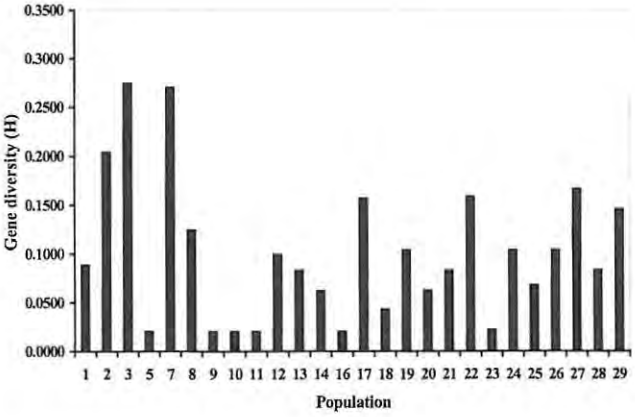


Figure 6.3: Histograms showing changes in proportions of polymorphic loci (PPL) and mean gene diversity values (H) in *S. capensis* and *S. serrata* along the coast from west to east. Division between Cape and Transkei coasts occurs between sites 15 and 16.

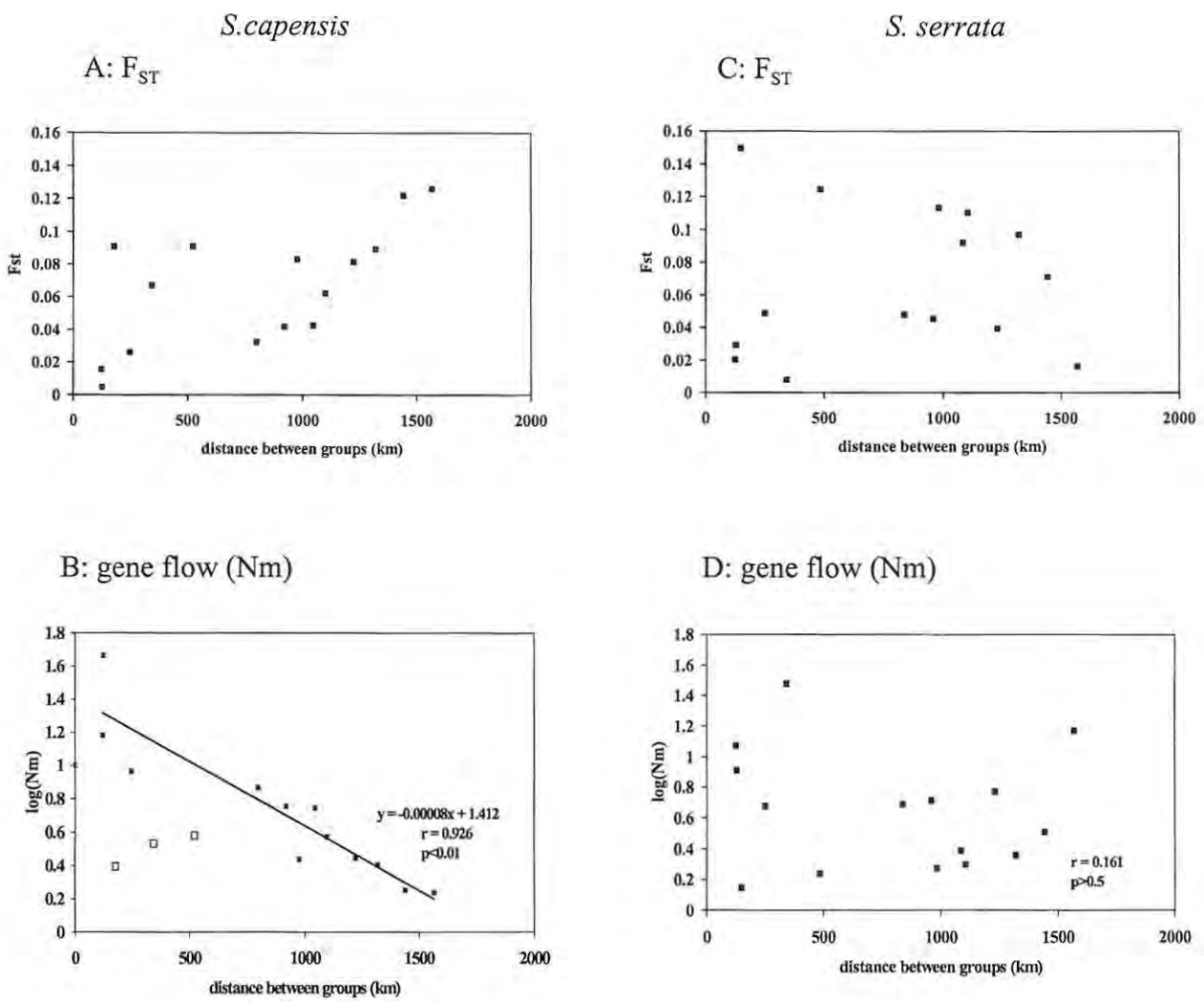


Figure 6.4: Scatterplots of F_{ST} and log(Nm) values with distance between groups of populations for both *S. capensis* and *S. serrata*. B: Hollow symbols represent data points which deviate from regression.

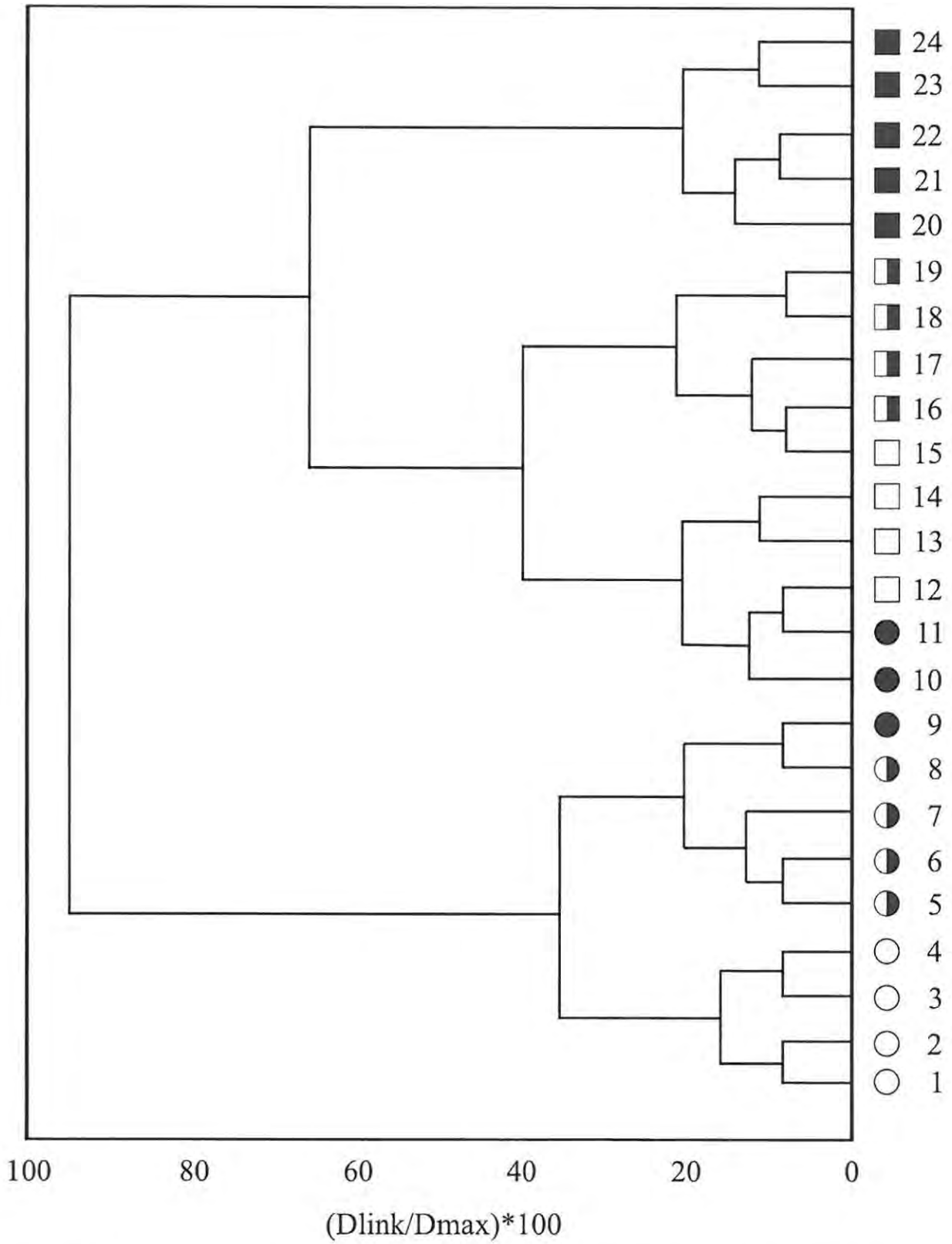


Figure 6.5: Dendrogram of *S. capensis* populations based on Nei's genetic distance values. Population symbols correspond to those shown on Figure 6.1.

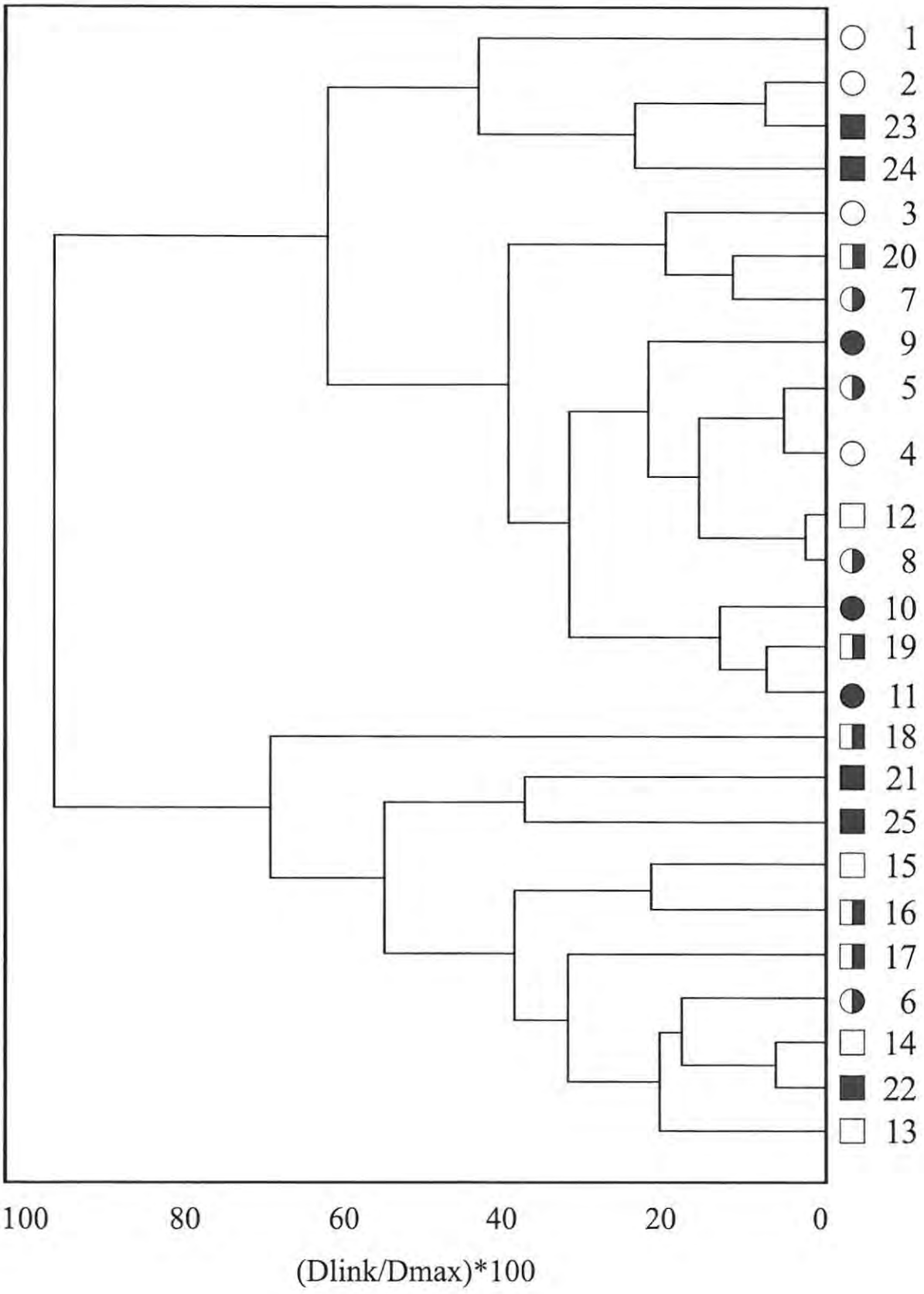


Figure 6.6: Dendrogram of *S. serrata* populations based on Nei's genetic distance values. Population symbols correspond to those shown on Figure 6.1.

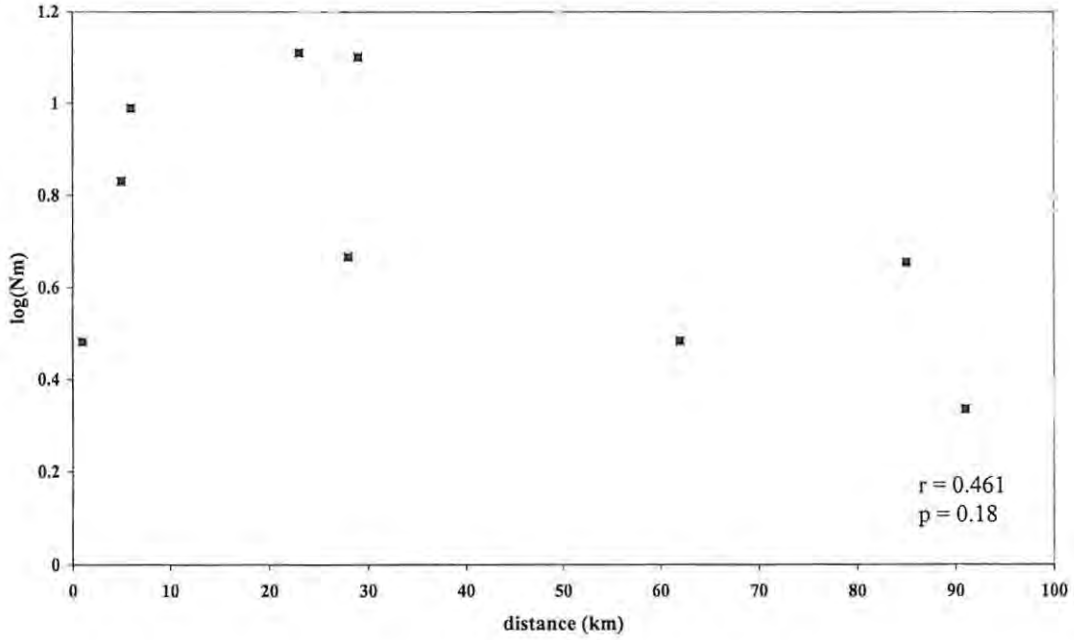


Figure 6.7: Scatterplot of log(Nm) values with distance between populations of *S. serrata* on a small scale.

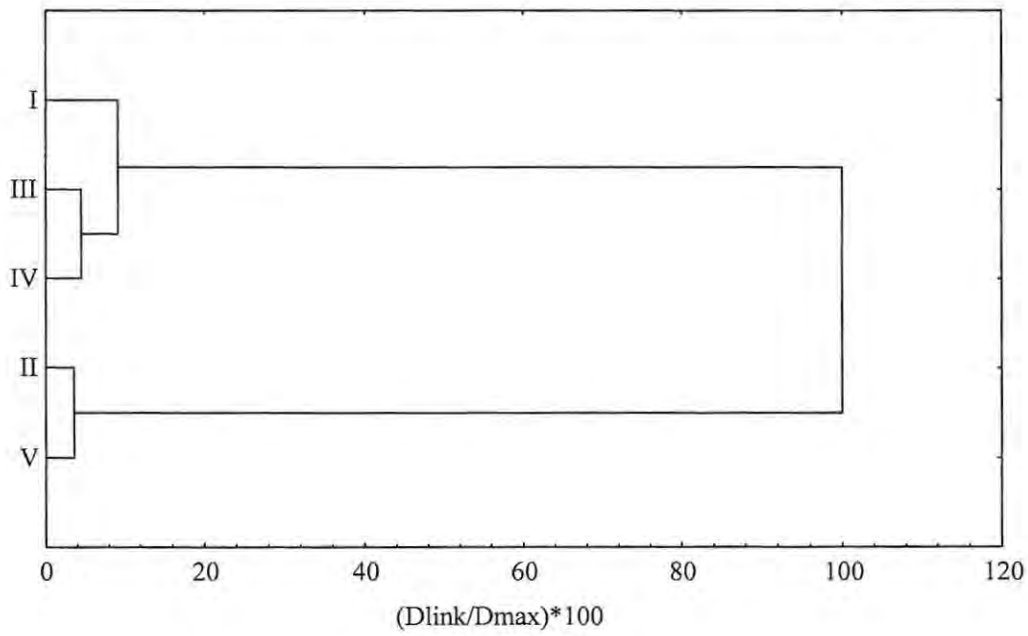


Figure 6.8: Dendrogram showing relationship between populations of *S. serrata* on a small scale.

CHAPTER 7: mtDNA sequencing

7.1 INTRODUCTION

Dispersal can result in high levels of gene flow throughout a species' range (Slatkin, 1985). This has been shown to be true for the canyon tree frog, *Hyla arenicolor* (Barber, 1999a and b), a small terrestrial snail, *Discus macclintocki* (Ross, 1999), a sedentary butterfly *Euphilotes enoptes* (Peterson, 1996) and the Mediterranean woodmouse *Apodemus sylvaticus* (Michaux *et al.*, 1996) to name but a few. Dispersal in marine benthic invertebrates is almost exclusively reliant on the presence of pelagic larvae within the life history. As discussed previously in detail, dispersal of pelagic larvae (followed by successful settlement and reproduction) and thus gene flow is known to occur over large distances. Through mtDNA sequencing studies, this has been shown in periwinkle snails *Littorina striata* (De Wolf *et al.*, 1998a) and *L. scutulata* (Kyle and Boulding, 2000), a sea cucumber, *Cucumaria miniata* (Arndt and Smith, 1998) and a gastropod *Crepidula depressa* (Collin, 2001). A sea urchin with pelagic larvae, *Echinothrix diadema* was found to disperse across the East Pacific Barrier, some 5000km (Lessios *et al.*, 1998). Limited dispersal, on the other hand, results in high levels of genetic variation among populations. High levels of population differentiation have been found in the periwinkle snail *Littorina subrotundata* (Kyle and Boulding, 2000), a gastropod *Crepidula convexa* (Collin, 2001) and an intertidal sea cucumber, *Cucumaria pseudocurata* (Arndt and Smith, 1998) all of which are direct developers.

Dispersal of any sort is often inhibited by the presence of a geographic barrier (Harrison, 1991; Shepherd *et al.*, 1992; Michaux *et al.*, 1996; Crease *et al.*, 1997; Barber, 1999a, 1999b; King *et al.*, 1999; Ross, 1999; Schizas *et al.*, 1999). In the marine environment such barriers are often hydrographic in nature (Hare *et al.*, 1994; Hare and Avise, 1996; Kojima *et al.*, 1997; Chase *et al.*, 1998; Lessios *et al.*, 1999), although a barrier may not be particularly obvious if it is ecological rather than physical in character (Lombard and Grant, 1986; Hellberg, 1998). Barriers have been found to limit dispersal and ultimately cause low levels of gene flow in several species, including the American oyster (Hare and Avise, 1996), the Japanese turban shell, *Turbo (Batillus) cornutus* (Kojima *et al.*, 1997), a benthic copepod, *Microarthridion littorale* (Schizas *et al.*, 1999), the limpet *Patella vulgata* round Britain (Hurst and Skibinski, 1995) and sea urchins of the genus *Eucidaris* (Lessios *et al.*, 1999). Detection of the existence of such a barrier requires examination of population genetics on a fine scale of resolution. A good candidate for such a study would be mtDNA sequencing.

To date, mtDNA sequencing has been extensively used in phylogenetic studies (e.g. Collins *et al.*, 1996; Reid *et al.*, 1996; Bastrop *et al.*, 1997; 1998; Ostellari *et al.*, 1998; Roe and Lydeard, 1998; King *et al.*, 1999; Koufopanou *et al.*, 1999; Rawson *et al.*, 1999; Distel, 2000; Lydeard *et al.*, 2000; Medina and Walsh, 2000; Wilding *et al.*, 2000) and is increasingly being used in population genetic studies (e.g. Hurst and Skibinski, 1995; Hare and Avise, 1996; Kojima *et al.*, 1997; Wilding *et al.*, 1997; Bastrop *et al.*, 1998; Chase *et al.*, 1998; Hellberg, 1998; Lessios *et al.*, 1998; Johnson and Bragg, 1999; Johnson, 2000; Kyle and Boulding, 2000; Turner *et al.*, 2000; Collin, 2001). Avise *et al.* (1987) argue that due to the almost ideal properties of the mtDNA molecule, this technique should be more extensively used in microevolutionary (population) studies. mtDNA is a small co-valently closed circular molecule of DNA, which is non-recombining. The gene arrangement is generally stable (although this is definitely not the case in

molluscs as shown by Wilding *et al.*, 1999) and inheritance is normally strictly maternal (Avise *et al.*, 1987; Harrison, 1991; Mitton, 1994) although this is not always true for molluscan mtDNA (Hoeh *et al.* 1996; Liu *et al.*, 1996; Quesada *et al.*, 1996). Maternal as well as paternal inheritance, known as doubly uniparental inheritance (DUI), where transmission of the mitochondrial type depends on the sex of the offspring (Skibinski *et al.*, 1994; Liu *et al.*, 1996), has been shown in marine and freshwater mussels (Hoeh *et al.*, 1996; Liu *et al.*, 1996; Quesada *et al.*, 1996). Presently, there is no evidence to suggest that DUI occurs in *Siphonaria*, however the possibility of this phenomenon occurring within the false limpets cannot be completely excluded.

mtDNA accumulates mutations much faster than nuclear loci and this allows for the examination of relationships within species at fine resolution (Harrison, 1991; Mitton, 1994). Due to this, the use of mtDNA sequencing in population genetic studies has developed into the broad field of phlyogeography (Bermingham and Moritz, 1998; Avise, 1998). Consequently, with the use of universal primers designed by Meriett *et al.* (1998), variation in the mitochondrial cytochrome *b* gene was used to investigate within- and among-population variation in *Siphonaria capensis* and *S. serrata*.

7.2 METHODS

Samples of both *Siphonaria capensis* and *S. serrata* were collected in two small areas along the Cape and Transkei coasts. Individuals were collected from the western area of the Cape coast, i.e. one individual each from populations 2, 3 and 4 and from the Transkei coast i.e. one individual each from populations 16, 30 and 31 and four individuals from population 32 for *S. capensis* (Figure 2.1, Chapter 2). For *S. serrata* one individual each from populations 2, 3, 4 and 5 were used from the Cape coast, while along the northeastern edge of the Transkei coast, one individual

each from populations 31 and 32 and three individuals from population 30 were used (Figure 2.1; Chapter 2). These sites were chosen to provide cost-effective and accurate within-population, among-population and between region comparisons of genetic variation between the two species using mtDNA sequencing.

7.2.1 DNA Extraction

Obtaining clean DNA samples from the foot muscle of these species was very difficult, even when additional CTAB (hexadecyltrimethylammonium bromide) was used in the extraction process. Previously, elevated levels of CTAB have been found to help remove polysaccharides when purifying DNA (Doyle and Doyle, 1990). Eventually, to overcome this problem, total cellular DNA was extracted from freshly frozen ovaries of individuals of both species. The concentration of mtDNA within a total DNA extraction is sufficient to be used for sequencing and the use of specific cytochrome *b* primers allows only the target sequence to be amplified using PCR (Hillis and Moritz, 1990; Neigel, 1997).

The ovary of each individual was removed and the total cellular DNA extracted using a modification of the hot CTAB method as described by Doyle and Doyle (1990). Each ovary was washed with distilled water, ground with a mortar and pestle and suspended in CTAB isolation buffer (3% CTAB, 1.4M NaCl, 0.2% 2-mercaptoethanol, 20mM EDTA, 100mM Tris-HCl pH8.0). Each sample was incubated at 60°C for two hours before an equal volume of phenol/chloroform-isoamyl alcohol (25:24:1) was added. The samples were shaken for 2 minutes and centrifuged at 10 000 rpm for 10 minutes. The supernatant was removed and mixed with an equal volume of chloroform-isoamyl alcohol (24:1). Again centrifugation was carried out at 10 000rpm for 10 minutes. The aqueous phase was removed and added to 2 volumes of ice-cold absolute ethanol and 10mM ammonium acetate. The DNA was allowed to precipitate overnight

at -5°C. Following centrifugation at 10 000rpm the DNA pellet was washed briefly with isopropanol, air-dried and finally resuspended in sterile water.

7.2.2 PCR Amplification

A section of the cytochrome *b* gene was amplified using a 50µl PCR reaction mixture containing PROMEGA Taq and buffer. Amplification was optimised over a range of magnesium chloride concentrations (1 to 4 mM). Universal primers designed by Merritt *et al.* (1998) were used in the initial amplification of this DNA sequence, the forward primer being: UCYTB151F: 5' TGT GGR GCN ACY GTW ATY ACT AA 3' and the reverse primer: UCYTB272R: 5' GCR AAN AGR AAR TAC CAY TC 3'. These primers are degenerate, thus allowing the primers to be used on a variety of molluscan species. The degenerate positions have the following ambiguity codes: N = A/C/G/T; R = A/G; W = A/T; Y = C/T. Following an initial denaturation stage of 4 minutes at 94°C, amplification was carried out for 40 cycles as follows: denaturation for 4 minutes at 94°C, primer annealing for 1 minute at 48°C and extension for 2 minutes at 72°C. A final extension stage of 6 minutes at 72°C concluded the PCR. Electrophoresis at 100V for 20 minutes of 10µl of the PCR products was carried out on 1% agarose gels in TBE buffer containing 1.5µl ethidium bromide (10µg/ml). Successful amplification was then visualised on an Ultra Violet transilluminator as a single band of DNA product per sample.

Approximately 200µl of amplified product was cleaned and concentrated into 20µl of water using a QIAGEN QIAQuick PCR Product Purification Kit. Sequencing reactions were then carried out using the ABI PRISM cycle sequencing kit according to the manufacturer's instructions. Initially, the segment of the cytochrome *b* gene was sequenced in both directions using primers UCYTB151F and UCYTB272R (Merritt *et al.*, 1998). Primer mismatch occurred and so additional forward and reverse internal primers were designed for each species;

"*S.capensis*-forward" (5' TTC TGC AAT TCC TTA (CT)AT TTG 3'), "*S. capensis*-reverse" (5' TAT AGG ATT AGC ATT AGT (AG)AA 3'), "*S.serrata*-forward" (5' ATC GGC TTT ACC TTT AGT AGG 3') and "*S. serrata*-reverse" (5' GT(CT) GAA TAT GTC TAG GTG TTA 3'). Sequencing was then carried out on an ABI3100 automated DNA sequencer.

7.2.3 Data Analysis

Component sequences from each PCR product were assembled, checked and edited using Sequencher version 3 (Gene Codes Corporation). The complete sequences were aligned manually using DAPSA (DNA And Protein Sequence Alignment; E H Harley, Department of Chemical Pathology, University of Cape Town). DAPSA was also used to generate data files for further analysis in PAUP version 4.0b3a (Swofford, 2000). This programme was used to generate neighbour joining trees of individuals within each species, with 1000 bootstrap replicates.

7.3 RESULTS

A total of 414 base pairs were sequenced in each species (Tables 7.1 and 7.2). Within *S. capensis* 29 variable characters were found of which nine were parsimony informative characters, i.e. characters found to vary between more than one individual. Within *S. serrata*, the direct developer, 50 variable characters were found of which almost half (23) were parsimony informative characters. This shows that approximately two thirds of the variable characters within *S. capensis* were unique while within *S. serrata* only half of the variable characters were unique to a particular individual.

7.3.1 *Siphonaria capensis*

A neighbour joining tree generated using PAUP version 4.0b3a (Swofford, 2000) revealed a distinct separation of the individuals from the Cape and Transkei coastal regions into two clades. This separation was supported by a bootstrap value of 100% (Figure 7.1A). Within the Cape coastal region, individuals from Melkbosstrand (2) and Sea Point (3) could not be distinguished. Within the Transkei regional clade, within population variation was greater (between individuals from population 32) than the level of variation among populations, for example 16-1 and 32-3 or 32-4 (Figure 7.1A).

7.3.2 *Siphonaria serrata*

In the direct developer, *S. serrata*, the greatest number of substitutions occurred between groups of individuals belonging to the two coastal regions, which were separated into two clades (Figure 7.1B). This separation was supported by a high bootstrap value of 100%. Within each of the coastal regions, a relatively high number of substitutions occurred among populations. Within the Transkei regional clade, the closest individuals were those belonging to the Shelly Beach population (30), i.e. there was low within-population variation.

7.3.3 Comparison between species

Clearly, there were more substitutions between clades from the two coastal regions in *S. serrata* than in *S. capensis* (Figure 7.1 A and B). This implies that within *S. serrata* there is less gene flow between the two coastal regions than within the pelagic developer, *S. capensis*. Within each coastal region, it is clear that there were a greater number of substitutions among populations in *S. serrata* than *S. capensis*. As predicted a low level of variation can be seen within *S. capensis* populations (i.e. population 32; Figure 7.1A).

7.4 DISCUSSION

Hierarchical analysis of the genetic variation within *S. capensis* and *S. serrata*, in the form of neighbour joining trees, confirmed the predictions that population genetic structure is influenced by mode of reproduction. *S. capensis*, with its potentially high dispersing pelagic larvae, was found to have high within-population variation and low among-population variation (Figure 7.1A). Interestingly, individuals from the two coastal regions formed distinct clades. This suggests that gene flow (through larval dispersal) diminished between the two extreme ends of the sampling region.

Siphonaria serrata, the direct developer, also conformed to the expectations that its limited dispersal potential would result in relatively low within-population variation and high among-population variation (Figure 7.1B). Most of the variation in this species was found to occur between the two coastal regions, and the separation of individuals from the two coastal regions was far greater in *S. serrata* than *S. capensis*.

While there is an abundance of literature using allozyme analysis to examine population genetic structure within virtually every type of organism (e.g. Nevo *et al.*, 1984), few studies have used mtDNA sequencing for this purpose (Collin, 2001). A few examples of mtDNA sequencing studies show similar results to those found in these *Siphonaria* species. Sea urchins belonging to the same genus, *Heliocidaris*, with differing developmental modes were found to conform to the prediction that the species with limited development showed a greater level of population differentiation (McMillan *et al.*, 1992). A comparison of two sea cucumber species also revealed no genetic structuring within the pelagic developer, *Cucumaria miniata* but high among-population variation within the direct developer, *C.*

pseudocurata (Arndt and Smith, 1998). A phylogeographic study of five species of *Crepidula* also clearly illustrated the proposed link between dispersal and population genetic structure (Collin, 2001). A study on four species of *Littorina* snails along the North American coast showed that one direct developer and one species with pelagic larvae conformed to predictions that the pelagic larvae would allow for greater gene flow through dispersal. However, *L. scutulata*, a planktonic developer, showed low but statistically significant population structure, illustrating that the relationship between larval dispersal potential and population genetic structure is far from simple and often influenced by extrinsic factors such as historical demography and oceanography (Kyle and Boulding. 2000).

The geographic differentiation illustrated among individuals of both species in this study has been seen in a number of other studies. mtDNA sequencing showed strong concordance between clades of the canyon tree frog, *Hlya arenicolor*, and geographic location (Barber, 1999b). The geographic structuring (phylogeography) of a limpet *Patella vulgata* (Hurst and Skibinski, 1995), the Japanese turban shell, *Turbo cornutus* (Kojima *et al.*, 1997), the pantropical sea urchin *Euciduaris* (Lessios *et al.*, 1999), a deep sea bivalve *Deminucula atacellana* (Chase *et al.*, 1998), a freshwater bivalve, *Lasmigona subviridis* (King *et al.*, 1999), a small terrestrial snail *Discus macclintocki* (Ross, 1999), benthic copepods, *Microarthridion littorale* (Schizas *et al.*, 1999) and the American oyster, *Crassostrea virginica* (Hare and Avise, 1996) were also easily observed through mtDNA sequencing. Geographic structuring is often attributed to some form of geographic barrier (Harrison, 1991; Shepherd *et al.*, 1992; Michaux *et al.*, 1996; Crease *et al.*, 1997; Barber, 1999a, 1999b; King *et al.*, 1999; Ross, 1999; Schizas *et al.*, 1999). In the marine environment, this usually occurs in the form of an hydrographic barrier (Hare and Avise, 1996; Kojima *et al.*, 1997; Chase *et al.*, 1998; Lessios *et al.*, 1999) or alternatively as an ecological barrier, which tends to be less

obvious (Hare and Avise, 1996; Hellberg, 1998). There is some evidence from *S. capensis* that a barrier limits pelagic larval dispersal along the South African coast. The individuals from the Cape coast and those from the Transkei coast form separate groups with the neighbour joining tree and the branch joining the two groups has a bootstrap value of 100% (Figure 7.1A). However, this separation of the individuals into their geographic regions may be artificial and due to the sampling strategy.

While some DNA research shows support for results found using allozyme analysis (e.g. Bastrop *et al.*, 1997; Wilding *et al.*, 1997; Lessios *et al.*, 1998; Kyle and Boulding, 2000), there is evidence that DNA studies are potentially more powerful and can increase the resolution of the differences between and within species as compared to allozyme analysis (Hillis and Moritz, 1990; Harrison, 1991; Mitton, 1994; Hurst and Skibinski, 1995; Wilding *et al.*, 2000). The results seen in this study of *S. capensis* and *S. serrata* reflect and confirm those found in the allozyme analysis. They lend support to the hypothesis that pelagic larvae of *S. capensis* do indeed allow for long distance dispersal along the coastline. However, the mtDNA sequencing results show some separation between individuals of *S. capensis* on a regional basis.

In 1987, Avise *et al.* (1987) advocated that through the near perfect properties of mtDNA, sequencing of genes within this molecule would provide invaluable research in the field of phylogeography (population genetics). Indeed, this study and others (e.g. Hurst and Skibinski, 1995; Avise, 1998; Klye and Boulding, 2000; Collin, 2001) more than prove their point. mtDNA sequencing allows for fine resolution of the relationships within a species (Mitton, 1994) and this leads to a greater understanding of the phylogenetics of a species, especially if there is strong geographic differentiation (Avise *et al.*, 1987). With the advent of universal

primers and the relatively cheap automated sequencing now available, this technique is set to become more widely used in the field of population genetics.

As stated by Collin (2001) a great many more studies such as this, utilising mtDNA sequencing for population studies of one than one species, are needed in the marine field. The technique allows for accurate, precise results that are easily comparable to other studies.

7.5 CONCLUSION

As predicted, the pelagic developer *S. capensis* showed high within-population variation and low among-population and between-region variation when compared to the direct developer, *S. serrata*. The two *Siphonaria* species examined in this study are from the same genus and were collected from the same sites, thus ensuring that they experienced similar ecological conditions. As the only major difference between them was their type of larval development and it can be conclusively stated that developmental mode is the underlying factor controlling the striking difference in the number of substitutions found to separate individuals from the two coastal regions.

Table 7.1: Cytochrome *b* sequence alignment of *S. capensis* individuals. Each individual is labelled according to the population to which it belongs and is given an unique number.

```
                                                                                                  60
2-1         TTGTGGAGCC ACTGTAATCA CGTAATCTTC TTTCTGCAAT TCCTTACATT GGACCTTGGG
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ------T--- ----------
16-1        ---------- ---------- -----C---- ---------- ---------- ----------
30-1        ---------- ---------- -----C---- ---------- ---------- -----C----
31-1        ---------A ----------T----C---- ---------- ---------- ----------
32-1        ---------- ---------- -----C---- ---------- ---------- ----------
32-2        ---------- ---------- -----C---- ---------- ---------- ----------
32-3        ---------- ---------- -----C---- ---------- ---------- ----------
32-4        ---------- ---------- -----C---- ---------- ---------- ----------

                                                                                                 120
2-1         TTGTTGAATG AGTTTGAGGG GGATTCTCTG TAGGGCAGTC GACTCTTAAT CGCTTTTTCT
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
16-1        -------G-- ---------- ---------- ---------- T--C------ ----------
30-1        ---------- ---------- ---------- ---------- ---C------ ----------
31-1        ---------- ---------- -------A-- ---------- T--C------ ----------
32-1        ---------- ---------- -------A-- ---------- T--C------ ----------
32-2        ---------- ---------- -------A-- ---------- T--C------ --------T-
32-3        ---------- ---------- ---------- ---------- T--C------ ----------
32-4        ---------- ---------- ---------- ---------- T--C------ ----------

                                                                                                 180
2-1         CCTTGCACTT TATTATACCG TTTCTGATTG GTGGTCTTTC AGGACTACAT GTTTTATTTC
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---T------ ---------- ---------- ----------
16-1        ---------- ---------- ---------- -G-------- ---------- ----------
30-1        ---------- ---------- ---------- -G-------- ---------- ----------
31-1        ---------- ---------- ---------- -G-------- ---------- ----------
32-1        ---------- ---------- ---------- -G-------- ---------- ----------
32-2        ---------- ---------- ---------- -G-------- ---------- ----------
32-3        ---------- ---------- ---------- -G-------- ---------- ----------
32-4        ---------- ---------- ---------- -G-------- ---------- ----------

                                                                                                 240
2-1         TTCATGAAAA AGGATCTACA AATCCTTTAG GGGAGTTAAA TCATGTAAGA AAAATTCCAT
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
16-1        ---------- ---------- ---------- ---------- ---------- ----------
30-1        ---------- ---------- ---------- ---------- ---------- ----------
31-1        ---------- ---------- ---------- ---------- ---------- ----------
32-1        ---------- ---------- ------A--- ---------T ---------- ----------
32-2        ---------- ---------- ---------- ---------- ---------- ----------
32-3        ---------- ---------- ---------- ---------- ---------- ----------
32-4        ---------- ---------- ---------- ---------- ---------- ----------
```

```
                                                                                                  300
2-1         TCCATCCTTA CTTTTCTTGA AAAGATATAG TGGGATTTGT TGTTTTATTT GTAATTCTTG
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
16-1        ---------- ---------- ---------- -A-------- ---------- ----------
30-1        ---------- ---------- ---------- -A-------- ---------- ----------
31-1        ---------- ---------- ---------- ---------- ---------- ----------
32-1        ---------- ---A------ ---------- -A-------- ---------- ----------
32-2        ---------- ---------- ---------- -A-------C ---------- ----------
32-3        ---------- ---------- ---------- -A-------- ---------- ----------
32-4        ---------- ---------- ---------- -A-------- ---------- ----------

                                                                                                  360
2-1         TTCTTTTAGG ATTTTTCTAC CCAACTTTGT TAGGTGATCC TGAAAACTTT CACTAATGCT
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------A ---------- ---------- ----------
16-1        ---------- G--------- ---------- -------C-- ---------- ----------
30-1        ---------- G--------- ---------- -------C-- ---------- ----------
31-1        ---------- G-----A--- ---------- -------C-- ---------- ----------
32-1        ---------- G--------T ---------- -------C-- ---------- G---------
32-2        ---------- G--------- ---------- -------C-- ----T----- ----------
32-3        ---------- G--------T ---------- -------C-- ---------- ----------
32-4        ---------- G--------T ---------- -------C-- ---------- ----------


2-1         AATCCTATAG TGACACCCGT ACACATTCAA CCAGAGTGGT ACTTCCTGTT CGCA
3-1         ---------- ---------- ---------- ---------- ---------- ----
4-1         ---------- ---------- ---------- ---------- ---------- ----
16-1        ---------- ---------- ---------- ---------- ---------- ----
30-1        ---------- ---------- ---------- ---------- ---------- ----
31-1        ---------- ---------- ---------- ---------- ---------- ----
32-1        ---------- ---------- ---------- ---------- ---------- ----
32-2        ---------A ---------- --T------- --C------- ---------- ----
32-3        ---------- ---------- ---------- ---------- ---------- ----
32-4        ---------- ---------- ---------- ---------- ---------- ----
```

Table 7.2: Cytochrome *b* sequence alignment of *S. serrata* individuals. Each individual is labelled according to the population to which it belongs and is then given an unique number.

```
                                                                                                  60
2-1         --TGTGGAGC CACTGTAATC ACTAATTTAC TATCGGCTTT ACCTTTAGTA GGTCCTTCAT
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
5-1         ---------- ------T--- ---------- ---------- ---------- --------G-
30-1        ---------- ---C------ ---------- ---------- ---------- ----------
30-2        ---------- ---------- ---------- ---------- ---------- ----------
30-3        A--------- ---------- ---------- ---------- ---------- -----C----
31-1        ---------- ---------- ---------- --------C- ---------- ----------
32-1        ---------- ---------- ---------- ---------- ---------- ----------

                                                                                                 120
2-1         TAGTTGAATG AGTATGAGGA GGATTTTGTG TTAGTGAAGC TTCACTAACA CGATTCTTTA
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
5-1         ---------- ---------- ---------- ---------- ---------- ----------
30-1        ---------- ---------- ---------- ---------- ---------- -----T----
30-2        ---------- ---------- ---------- ---------- ---------T -----T----
30-3        ---------- ---------- ---------- ---------- ---------- -----T----
31-1        ---------- ---------- ---------- ---------- ---------- ----------
32-1        ---A----A- ---------- ---------- ---------- ---------- ----------

                                                                                                 180
2-1         CTTTACATTT TCTTCTTCCA TTTACTATTC TAGCAAGTTC AGGGTTACAT CTTTTATTTT
3-1         ---------- ---------- ---------- ---------- ---------- --A-------
4-1         ---------- ---------- ---------- ---------- ---A------ ----------
5-1         ---------- ------C--- ---------- ---------- ---------- ----------
30-1        ---------- ------C--- ---------- ---------- ---A------ ----------
30-2        ---------- ------C--- ---------- ---------- ---A------ ----------
30-3        ---------- ------C--- ---------- ---------- ---A------ ----------
31-1        ---------- ------C--- ---------- ---------- ---A-G---- ----------
32-1        ---------- ------C--- ---------- ---------- ---A------ ----------

                                                                                                 240
2-1         TACATGATAA AGGGTCTTCT AATCCTTTAG GATTAATAAA TAATATTAAT AAAAATAGAT
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- --------C- ---------- ----------
5-1         ---------- ---------- ---------- --------T- ---------- ----C-----
30-1        ---------- ---------- ---------- ---------- ---------- ----C-----
30-2        ---------- ---------- ---------- ---------- ---------- ----C-----
30-3        ---------- ---------- ---------- ---------- ---------- ----C-----
31-1        ---------- ---------- ---------- ---------- ---------- ----C-----
32-1        ---------- ---------- ---------- ---------- ---------- ----C-----

                                                                                                 300
2-1         TTCATCCATA CTTTACATGA AAGGATATTG TAGGTTTTAG TATGCTTGGA GTATTTTTTG
3-1         ---------- ---------- ---------- ---------- ---------- ----------
4-1         ---------- ---------- ---------- ---------- ---------- ----------
5-1         ---------- ---------- ---C------ ---------- -G-------- -----A----
30-1        ---------- ---------- ---------- ---------- ----T----- ----------
30-2        ---------- ----C----- ---------- ---------C ---------- ----------
30-3        ---------- ---------- ---------- ---------- ----T----- ----------
31-1        ---------- ---------- ---------- ---------- -C-T------ ----------
32-1        ---------- ---------- ---------- ---------- ----T----- ----------
```

```
                                                                                                                  360
2-11        ATGGAGTAAC TTTTTGTTAT CCTGCTCTTG TACAAGAGCA CTCAAATTTT ATTCAAGCAG
3-1         ---------- A--------- ---------- -------AAC ---------- ----------
4-1         ---------- ---------- ---------- -G----AA-C ---------- ----------
5-1         ---------G A--------- ---------C ---------- ---------- ----------
30-1        TCCTG---G- -----T---- ---------C ---------C -CA------- ----------
30-2        TCCTG---G- -----T---- ---------C ---------C -CA------- ----------
30-3        TCCTG---G- -----T---- ---------C ---------C -CA------- ----------
31-1        TCCTG----- A----T---- ---------C ----G-G--C -CA------- ----------
32-1        TCCTG---G- A----T---- ---------C ---------C -CA------- ----------


2-1         ACTGCTTAAA CAACACCTAG ACATATTCAA CCTGATTGGT ATTTCCTCTT CGCA
3-1         ---------- --C------C ---------- ---------- ---------A ----
4-1         ---------- --C------- ---------- ---------- ---------- ----
5-1         ---------- T--------- ---------- ---------- G--------- ----
30-1        --A------- ---------- ---------- -----G---- ---------- ----
30-2        --A------- T--------- ---------- -----G---- -------G-- ----
30-3        --A------- T--------- ---------- -----G---- ---------- ----
31-1        --A------- T--------- ---------- -----G---- A------G-- ----
32-1        --A-----G- T--------- ---------- -----G---- A--------- ----
```

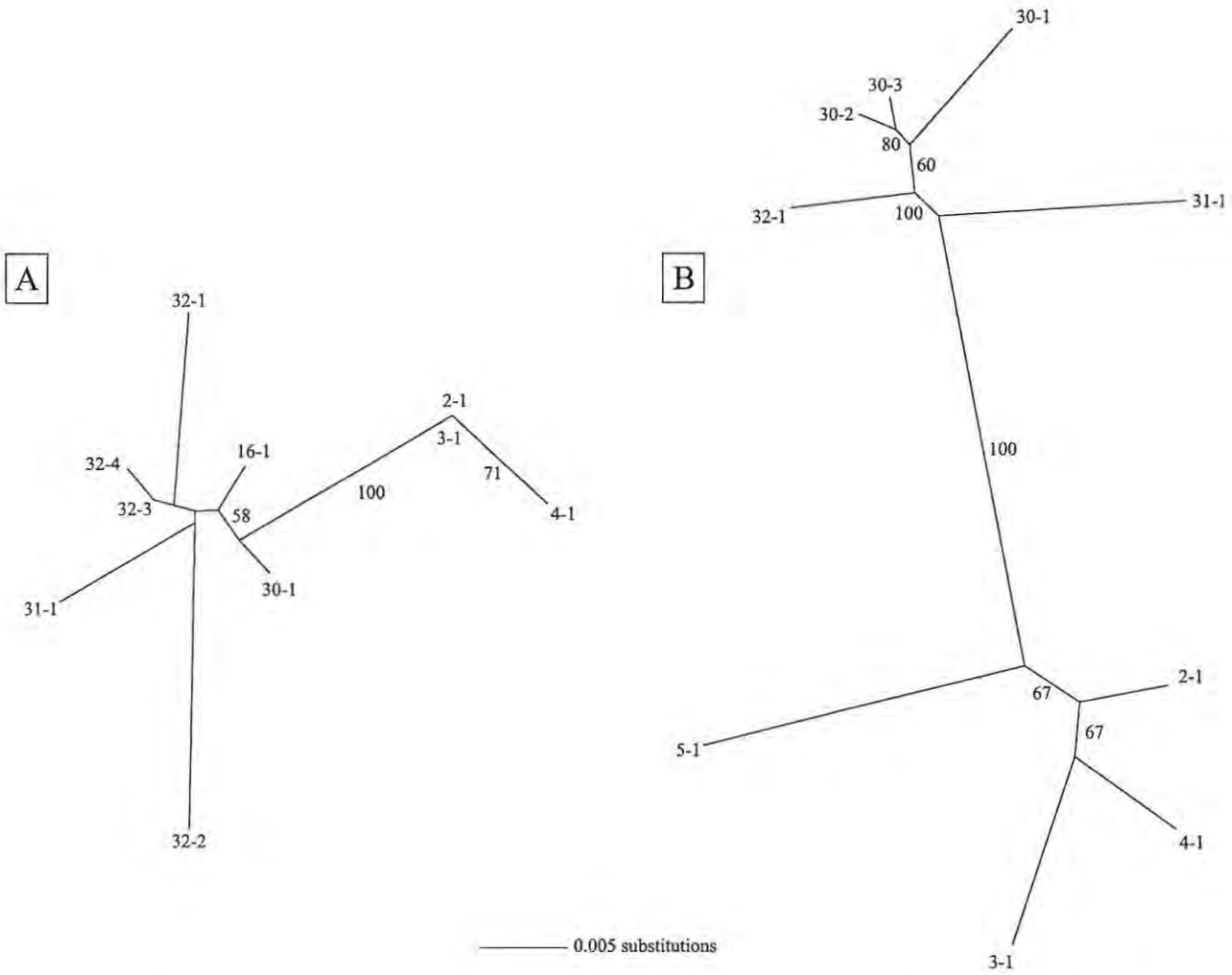


Figure 7.1: Neighbour-joining trees of individuals of *S. capensis* (A) and *S. serrata* (B) based on a 414bp region of the cytochrome *b* gene. The trees are drawn to the same scale as indicated by the scale bar. Numbers at branch tips represent each individual sampled, the first number indicates the population of origin and the second number is unique to each individual from that population. Numbers along branches represent bootstrap values from 1000 replicates. Absence of a bootstrap value indicates that the branch was unresolved.

CHAPTER 8: General Discussion

Dispersal lies at the centre of understanding a number of processes, such as ecological relationships, behavioural interactions, evolutionary potential (Knowlton and Keller, 1986) and, of course, population genetic structure. Dispersal may be achieved actively by organisms (Knowlton and Keller, 1986), but in the marine field dispersal of larvae most often occurs passively and is generally under the influence of environmental factors, especially ocean currents (Olson, 1985; Shepherd *et al.*, 1992; Palumbi, 1995; Hellberg, 1998; Hilbish *et al.*, 2002). Dispersal of marine benthic invertebrates is strongly affected by life history strategy (Scheltema, 1971 and 1975; Olson, 1985; Gaines and Bertness, 1992; Bohonak, 1999). The relationship between life history strategy and population genetic structure has been examined and debated for decades (Hedgecock, 1986; Johannesson and Johannesson, 1990; Liu *et al.*, 1991; Williams and Benzie, 1996; Swearer *et al.*, 1999). Biologists have come to understand that this is not a simple relationship but rather it involves many confounding factors.

Comparative studies of genetic structure within species have become popular over recent years. Such studies allow a better interpretation of the mechanisms operating within each species than if only a single species is examined (Bohonak, 1999; Kyle and Boulding, 2000; Collin, 2001). This study is no exception. Through various indirect techniques (most notably variation in shape through morphometrics, allozyme analysis and mtDNA sequencing), it has been shown that, as predicted, the pelagic larvae of *Siphonaria capensis* allow for long distance dispersal and extensive gene flow. This, in turn, results in high within-population

variation and low among-population variation. Clearly, high levels of gene flow in *S. capensis* do not allow for the establishment of any particular gene combinations (Berglund and Lagercrantz, 1983; Slatkin, 1985). In contrast, the direct development of *S. serrata* limits dispersal and results in low genetic variation within populations and high levels of genetic variation among populations, though there appears to be sufficient gene flow among populations of *S. serrata* to maintain genetic cohesion of even quite distinct populations (see details in Chapter 6). Thus, gene flow is not uncommon among populations of *S. serrata* and, as discussed by Slatkin (1981), even a low level of gene flow will effectively unify a species.

Such a contrast between species has been observed in a number of studies on closely related species whose major difference lies in the mode of reproduction. Examples include, periwinkles snails (Janson, 1987; Johannesson, 1988; Kyle and Boulding, 2000), sponge-dwelling shrimps (Duffy, 1993), solitary corals (Hellberg, 1996), tropical sea anemones (Russo *et al.*, 1994), starfish (Hunt, 1993) and two species of the prawn *Palaemon* (Berglund and Lagercrantz, 1983).

The main conclusions reached regarding population genetic structure within species is given in Table 8.1. Morphological variation in *S. capensis* and *S. serrata* was found to be complex. Patterns of variation in size were similar between the two species and largely under the control of environmental factors. Primary productivity (possibly influenced by the level of exposure on the shore) is probably the main cause for such morphological variation in size along the coast; size decreases from west to east, as does primary production. Variation in shape was found to vary as we would predict if the phenotype was mainly under genetic control. This illustrates that, with careful interpretation of multivariate analyses, morphological variation can reveal information about the genetic structure of populations.

Detailed analysis of the morphological variation in these limpets provided a good indication of the interaction between genotype and the environment in producing the observed phenotypic variation (Chapter 4).

Disappointingly, PAGE of total soluble proteins did not show any notable differences between the two pulmonate limpet species. Levels of within- and among-population variation of both species were found to be similar. While quick and inexpensive (Nxomani *et al.*, 1994; Brown, 1999), this technique is not very useful for comparing genetic structure within these two closely related species. This may be due to the lack of a strong genetic influence on the total soluble proteins (Burton, 1983).

Allozyme analysis clearly illustrated differences in population genetic structure between *S. capensis* and *S. serrata*. This technique has been widely used (Hillis and Moritz, 1990; Amos and Hoelzel, 1992) and the statistical analysis of the results is particularly robust (Neigel, 1997; J W H Ferguson, *pers comm*). This technique did not require any prior genetic information of the two species and clearly showed that larval dispersal in *S. capensis* does affect population genetic structure, resulting in high within-population variation and low among-population variation. Added to this, it was possible to analyse statistically the relationship between estimated gene flow within each species and the ocean currents. Despite a large number of possible confounding factors, a highly significant multiple regression showed that ocean currents influenced larval dispersal and gene flow in *S. capensis*. The strong, near shore Agulhas Current along the Transkei coast assists in long distance larval dispersal resulting in high gene flow in that region. The weak, highly variable, wind driven inshore currents along the Cape coast reduce larval dispersal, and gene flow was significantly lower in this region.

mtDNA sequencing has only been utilised as a tool for examining population genetic structure for the last decade or so (Avise *et al.*, 1987; Bermingham and Moritz, 1998). None the less it is clearly very useful. This technique is unlikely to be affected by selection (Hillis and Moritz, 1990) and is a pure examination of genetic variation in individuals. Using relatively few samples this technique easily illustrated the predicted differences between the two *Siphonaria* species, revealing a higher level of gene flow within *S. capensis*.

Several authors advocate the importance of using more than one marker when conducting research on population genetics (Slatkin, 1985; Hillis and Moritz, 1990; Beaumont *et al.*, 1993; Mitton, 1994; Neigel, 1997; Bohonak, 1999; Esselman *et al.*, 1999). This study clearly illustrates such a need. Each technique highlights a unique aspect of the genetic structure of these two species and together the different analyses give a clear view on the complex nature of population genetic structure in *S. capensis* and *S. serrata*. Firstly, variation in size within both species was found to be similar and largely under the influence of environmental factors, most probably wave exposure and primary production. Variation in shape was found to be under stronger genotypic influence (Chapter 4). Secondly, dispersal of the pelagic larvae of *S. capensis* was not only influenced by distance between populations along the coast, but was also strongly influenced by ocean currents. This was seen in a multiple regression between levels of gene flow and distance between populations, as well as distance of the main Agulhas Current from the shore (Chapter 6). Several studies in the world's major oceans have shown a positive link between dispersal and ocean currents (Scheltema, 1975; Hunt, 1993; Williams and Benzie, 1996; Brierley and Brandon, 1999; Darling *et al.*, 2000; Uthicke *et al.*, 2001). The strong inshore Agulhas Current along the Transkei coast was found to result in a relatively high level of gene flow in both species. This current probably also assisted the

alternative dispersal strategies of *S. serrata*. In contrast, the inshore currents along the Cape coast are relatively weak and highly variable and this, along with the high number of bays on the coast, resulted in lower levels of gene flow within *S. capensis*. Finally, the mtDNA sequencing data showed that individuals from the different coastal regions, i.e. Cape and Transkei coasts, were more different from one another in the direct developer, *S. serrata* than in the pelagic developer, *S. capensis*.

While gene flow is certainly linked to dispersal potential within *S. capensis* and *S. serrata*, this may not be true for every marine species (eg *Littorina striata*; De Wolf *et al.*, 1998b and c). These two pulmonate limpet species represent opposite ends of the scale of life history strategies occurring in marine invertebrates. The high dispersal potential of the pelagic larvae of *S. capensis* is realised and is assisted by the ocean currents around the coast of South Africa. The gene flow within *S. capensis* effectively unifies the species, preserving the cohesiveness and integrity of the species (Hedgecock, 1986) and also prevents localised adaptation (Slatkin, 1981). The low dispersal potential of *S. serrata* results in low levels of gene flow, which, nevertheless, is still sufficient to unify the species (Slatkin, 1981).

Table 8.1: Summary of conclusions drawn from different molecular techniques used to analyse genetic structure within *S. capensis* and *S. serrata*. **Bold text** represents results, which did not conform to the predictions made.

	S. capensis		*S. serrata*	
	within population variation	among population variation	within population variation	among population variation
Predictions	high	low	low	high
Morphometric analysis	**size: similar** shape: high	**similar** low	**size: similar** shape: low	**similar** high
PAGE of total soluble proteins	high	low	**high**	**low**
Allozyme analysis	high	low	low	high
mtDNA analysis	high	low	low	high

REFERENCES

Adamkewiez SL, Harasewych MG (1994) Use of random amplified polymorphic DNA (RAPD) markers to assess relationships among beach clams of the genus *Donax*. The Nautilus 2: 51-60.

Allanson BR (1959) On the systematics and distribution of the molluscan genus *Siphonaria* in South Africa. Hydrobiologia 12: 149-180.

Allcock AL, Brierley AS, Thorpe JP, Rodhouse PG (1997) Restricted gene flow and evolutionary divergence between geographically separated populations of the Antarctic octopus *Pareledone turqueti*. Marine Biology 129: 97-102.

Amos B, Hoelzel AR (1992) Applications of molecular genetic techniques to the conservation of small populations. Biological Conservation 61: 133-144.

Andersen H, Birkelund S, Christiansen G, Freundt E, (1987) Electrophoretic analysis of proteins from *Mycoplasma hominis* strains detected by SDS-PAGE, two-dimensional gel electrophoresis and immunoblotting. Journal of General Microbiology 133: 181-191.

Apostol BL, Black IV WC, Miller BR, Reiter P, Beaty BJ (1993) Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*. Theoretical and Applied Genetics 86: 991-1000.

Armbruster G (1995) Univariate and multivariate analyses of shell variables within the genus *Cochlicopa* (Gastropoda: Pulmonata: Cochlicopidae). Journal of Molluscan Studies 61: 225-235.

Armbruster G (1997) Evaluations of RAPD markers and allozyme patterns: evidence for morphological convergence in the morphotype of *Cochlicopa lubricella* (Gastropoda: Pulmonata: Cochlicopidae). Journal of Molluscan Studies 63: 379-388.

Arnaud JF, Daguzan J (1999) Spatial differentiation of allozyme frequencies in a subdivided population of the land snail *Helix aspersa*. Journal of Molluscan Studies 65: 267-271.

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Molecular Ecology 7: 1053-1064.

Arnheim N, White T, Rainey WE (1990) Application of PCR: organismal and population biology. Bioscience 40: 174-182.

Atkinson WD, Newbury SF (1984) The adaptations of the rough winkle, *Littorina rudis*, to desiccation and to dislodgement by wind and waves. Journal of Animal Ecology 53: 93-105.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of. Ecology and Systematics 18: 489-522.

Avise, JC (1998) The history and preview of phylogeography: a personal reflection. Molecular Ecology 7: 371-379.

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier Reef, Australia. Marine Ecology Progress Series 159: 175-187.

Baltanas A, Namiotko T, Danielopol DL (2000) Biogeography and disparity within the genus *Cryptocandona* (Crustacea, Ostracoda). Vie et Milieu 50: 297-310.

Barber PH (1999a) Phylogeography of the canyon treefrog, *Hyla arenicolor* (Cope) based on mitochondrial DNA sequence data. Molecular Ecology 8: 547-562.

Barber PH (1999b) Patterns of gene flow and population genetic structure in the canyon treefrog, *Hyla arenicolor* (Cope). Molecular Ecology 8: 563-576.

Barbieri M, Bavestrello G, Sara M (1995) Morphological and ecological differences in two electrophoretically detected species of *Cliona* (Porifera, Demospongiae). Biological Journal of the Linnean Society 54: 193-200.

Bardakci F, Skibinski DOF (1994) Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity 73: 117-123.

Bastrop R, Jurss K, Sturmbauer C (1998) Cryptic species in a marine polychaete and their independent introduction from North America to Europe. Molecular Biology and Evolution 15: 97-103.

Bastrop R, Rohner M, Sturmbauer C, Jurss K (1997) Where did *Marenzelleria* spp. (Polychaeta: Spionidae) in Europe come from? Aquatic Ecology 31: 119-136.

Beaumont AR, Morvan C, Huelvan S, Lucas A, Ansell AD (1993) Genetics of indigenous and transplanted populations of *Pecten maximus*: no evidence for the existence of separate stocks. Journal of Experimental Marine Biology and Ecology 169: 77-88.

Beckley LE (1983) Sea-surface temperature variability around Cape Recife, South Africa. South African Journal of Science 79: 436-438.

Beckley LE (1997) Distribution and dispersal of fish larvae. Oceanographic Research Institute Special Publication (ORI) Durban 140: 28-29.

Beckley LE, Hewitson JD (1994) Distribution and abundance of clupeoid larvae along the east coast of South Africa in 1990/91. South African Journal of Marine Science 14: 205-212.

Begon M, Harper JL, Townsend CR (1990) Ecology. individuals, populations and communities. 2nd Edition. Blackwell Scientific Publications, London. p 945.

Behrens Yamada S (1977) Geographic range limitation of the intertidal gastropods *Littorina sitkana* and *L. planaxis*. Marine Biology 39: 61-65.

Behrens Yamada S (1989) Are direct developers more locally adapted than planktonic developers? Marine Biology 103: 403-411.

Benzie JAH, Williams ST (1995) Gene flow among giant clam (*Tridacna gigas*) populations in Pacific does not parallel ocean circulation. Marine Biology 123: 781-787.

Benzie JAH, Williams ST (1997) Genetic structure of giant clam (*Tridacna maxima*) populations in the west Pacific is not consistent with dispersal by present-day ocean currents. Evolution 51: 768-783.

Berg DJ, Cantonwine EG, Hoeh WR, Guttman SI (1998) Genetic structure of *Quadrula quadrula* (Bivalvia: Unionidae): little variation across large distances. Journal of Shellfish Research 17: 1365-1373.

Berger EM (1973) Gene-enzyme variation in three sympatric species of *Littorina*. Biological Bulletin 145: 83-90.

Berglund A, Lagercrantz U (1983) Genetic differentiation in populations of two *Palaemon* prawn species at the Atlantic east coast: does gene flow prevent local adaptation? Marine Biology 77: 49-57.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. Molecular Ecology 7: 367-369.

Berry AJ (1977) Gastropoda: Pulmonata. In: Reproduction of Marine Invertebrates. Volume IV. (Eds: Giese AC, Pearse JS) Academic Press, New York, p181-226.

Bertness MD, Gaines SD (1993) Larval dispersal and local adaptation in acorn barnacles. Evolution 47: 316-320.

Beyermann B, Nurnberg P, Weihe A, Meixner M, Epplen JT, Borner T (1992) Fingerprinting plant genomes with oligonucleotide probes specific for simple repetitive DNA sequences. Theoretical and Applied Genetics 83: 691-694.

Bingham BL, Young CM (1991) Larval behaviour of the ascidian *Ecteinascidia turbinata* Herdman; an in situ experimental study of the effects of swimming on dispersal. Journal of Experimental Marine Biology and Ecology 145: 189-204.

Black R, Johnson MS (1981) Genetic differentiation independent of intertidal gradients in the pulmonate limpet *Siphonaria kurracheensis*. Marine Biology 64: 79-84.

Blanchetot A (1992) DNA fingerprinting analysis in the solitary bee *Megachile rotundata*: variability and nest mate genetic relationships. Genome 35: 681-688.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. The Quarterly Review of Biology 74: 21-45.

Bookstein FL (1989) "Size and shape": A comment on semantics. Systematic Zoology 38: 173-180.

Borges TC (1995) Discriminant analysis of geographic variation in hard structures of *Todarodes sagittatus* from the North Atlantic. ICES marine Science Symposium 199, 433-440.

Boulding EG, Buckland-Nicks J, Van Alstyne KL (1993) Morphological and allozyme variation in *Littorina sitkana* and related *Littorina* species from the Northeastern Pacific. The Veliger 36: 43-68.

Branch GM (1981) The biology of limpets: physical factors, energy flow, and ecological interactions. Oceanography and Marine Biology: an Annual Review 19: 235-380.

Branch GM, Branch M (1992) The living shores of southern Africa. Struik Publishers, Cape Town. p272.

Brandwood A (1985) The effects of environment upon shell construction and strength in the rough periwinkle *Littorina rudis* Maton (Mollusca: Gastropoda). Journal of Zoological Society of London 206: 551-565.

Brendonck L, De Meester L, Riddoch BJ (2000) Regional structuring of genetic variation in short-lived rock pool populations of *Branchipodopsis wolfi* (Crustacea: Anostraca). Oecologia 123: 506-515.

Brierley AS, Brandon MA (1999) Potential for long-distance dispersal of *Euphausia crystallorophias* in fast current jets. Marine Biology 135: 77-82.

Brown PC (1992) Spatial and seasoanl variation in chlorophyll distribution in the upper 30m of the photic zone in the southern Benguela/Agulhas ecosystem. South African Journal of Marine Science 12: 515-525.

Brown SA (1999) Genetic variation within and between some taxa of rare Cape proteaceae and the implications for their conservation. Phd thesis, Rhodes University.

Bucklin A, Marcus NH (1985) Genetic differentiation of populations of the planktonic copepod *Labidocera aestiva*. Marine Biology 84: 219-224.

Bulnheim H-P, Scholl A (1986) Genetic differentiation between populations of *Talitrus saltator* and *Talorchestia deshayesii* (Crustacea: Amphipoda) from coastal areas of the north-western European continent. Marine Biology 92: 525-536.

Burton RS (1983) Protein polymorphisms and genetic differentiation of marine invertebrate populations. Marine Biology Letters 4: 193-206.

Burton RS, Fledman MW, Curtsinger JW (1979) Population genetics of *Tigriopus californicus* (Copepoda: Haracticoida): I. Population structure along the central California coast. Marine Ecology Progress Series 1: 29-39.

Bustamante RH, Branch GM, Eekhout S (1995) Maintenance of an exceptional intertidal grazer biomass in South Africa: sibsidy by subtidal kelps. Ecology 76:2314-2329.

Caetano-Anolles G, Bassam BJ, Gresshoff PM (1992) Primer-template interactions during DNA amplification fingerprinting with single arbitrary oligonucleotides. Molecular Genetics 235: 157-165.

Cao W, Scoles G, Huel P; Chibbar RN (1999) The use of RAPD analysis to classify *Triticum accessions*. Theoretical and Applied Genetics 98: 602-607.

Chakir M, David JR, Pla E, Capy P (1995) Genetic basis of some morphological differences between temperate and equatorial populations of *Drosophila melanogaster*. Experientia 51: 744-748.

Chalmers KJ, Waugh R, Sprent JI, Simons AJ, Powell W (1992) Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity 69: 465-472.

Chambers RJ, McQuaid CD (1994a) Notes on the taxonomy, spawn and larval development of South African species of the intertidal limpet *Siphonaria* (Gastropoda: Pulmonata). Journal of Molluscan Studies 60: 263-275.

Chambers RJ, McQuaid CD (1994b) A review of larval development in the intertidal limpet genus *Siphonaria* (Gastropoda: Pulmonata). Journal of Molluscan Studies 60: 415-423.

Chambers RJ, McQuaid CD, Kirby R (1996) Determination of genetic diversity of South African intertidal limpets (Gastropoda: Siphonaria) with different reproductive modes using polyacrylamide gel electrophoresis of total cellular proteins. Journal of Experimental Marine Biology and Ecology 201: 1-11.

Chambers RJ, McQuaid CD, Kirby R (1998) The use of randomly amplified polymorphic DNA to analyze the genetic diversity, the systematic relationships and the evolution of intertidal limpets, *Siphonaria spp*. (Pulmonata: Gastropoda), with different reproductive modes. Journal of Experimental Marine Biology and Ecology 227: 49-66.

Chambers SM (1978) An electrophoretically detected sibling species of "*Goniobasis floridensis*" (Mesogastropoda: Pleuroceridae). Malacologia 17: 157-162.

Chase MR, Etter RJ, Rex MA, Quattro JM (1998) Bathymetric patterns of genetic variation in a deep-sea protobranch bivalve, *Deminucula atacellana*. Marine Biology 131: 301-308.

Christensen MS (1980) Sea-surface temperature charts for Southern Africa, south of 26°S. South African Journal of Science 76: 541-546.

Clark AG, Lanigan CMS (1993) Prospects for estimating nucleotide divergence with RAPDs. Molecular Biology and Evolution 10: 1096-1111.

Collin R (2001) The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Molecular Ecology 10: 2249-2262.

Collins TM, Frazer K, Palmer AR, Vermeij GJ, Brown WM (1996) Evolutionary history of northern hemisphere *Nucella* (Gastropod, Muricidae): molecular, morphological, ecological and paleontological evidence. Evolution 50: 2287-2304.

Cook LM (1983) Polymorphism in a mangrove snail in Papua New Guinea. Biological Journal of the Linnean Society 20: 167-173.

Corbet SW, Grant WS, Robinson TJ (1994) Genetic divergence in South African wildebeest: analysis of allozyme variability. Journal of Heredity 85: 479-483.

Correll JC, Rhoads DD, Guerber JC (1993) Examination of mitochondrial DNA restriction fragment length polumorphisms, DNA fingerprints, and randomly amplified polymorphic DNA of *Colletotrichum orbiculare*. Phytopathology 83: 1199-1204.

Craddock C, Lutz RA, Vrijenhoek RC (1997) Patterns of dispersal and larval development of archaeogastropod limpets at hydrothermal vents in the eastern Pacific. Journal of Experimental Marine Biology and Ecology 210: 37-51.

Crease TJ, Lee S-K, Yu S-L, Spitze K, Lehmans N, Lynch M (1997) Allozyme and mtDNA variation in populations of the *Daphnia pulex* complex from both sides of the Rocky Mountains. Heredity 79: 242-251.

Crossland S, Coates D, Grahame J, Mill PJ (1993) Use of random amplified polymorphic DNAs (RAPDs) in separating two sibling species of *Littorina*. Marine Ecology Progress Series 96: 301-305.

Crothers JH (1992) Shell size and shape variation in *Littorina littorea* (L.) from west Somerset. In: Proceedings of the Third International Symposium on Littorinid Biology. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p91-97.

Crouau-Roy B (1988) Genetic structure of cave-dwelling beetles populations: significant deficiencies of heterozygotes. Heredity 60: 321-327.

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row. New York.

Cunningham CO, Mo TA (1997) Random amplified polymorphic DNA (RAPD) analysis of three Norwegian *Gyrodactylus salaris* populations (Monogenea; Gyrodactylidae). The Journal of Parasitology 83: 311-314.

Dahle G, Rahman M, Eriksen AG (1997) RAPD fingerprinting used for discriminating among three populations of Hilsa shad (*Tenualosa ilisha*). Fisheries Research 32: 263-269.

Dai C-F, Fan T-Y, Yu J-K (2000) Reproductive isolation and genetic differentiation of a scleractinian coral *Mycedium elephantotus*. Marine Ecology Progress Series 201: 179-187.

D'Amato ME, Corach D (1997) Population genetic structure in the fresh-water anomuran *Aegla jujuyana* by RAPD analysis. Journal of Crustacean Biology 17: 269-274.

Darling KF, Wade CM, Stewart IA, Kroon D, Dingle R, Leigh Brown AJ (2000) Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. Nature 405: 43-47.

De Vienne D, Burstin J, Gerber S, Leonardi A, Le Guilloux M, Murigneux A, Beckert M, Bahrman N, Damerval C, Zivy M (1996) Two-dimensional electrophoresis of proteins as a source of monogenic and codominant markers for populations genetics and mapping the expressed genome. Heredity 76: 166-177.

De Wolf H, Backeljau T, Van Dongen S, Verhagen R (1998a) Large-scale patterns of shell variation in *Littorina striata* planktonic developing periwinkle from Macaronesia (Mollusca: Prosobranchia). Marine Biology 131: 309-317.

De Wolf H, Backeljau T, Verhagen R (1998b) Congruence between allozyme and RAPD data in assessing macrogeographical genetic variation in the periwinkle *Littorina striata* (Mollusca, Gastropoda). Heredity 81: 486-492.

De Wolf H, Backeljau T, Verhagen R (1998c) Spatio-temporal genetic structure and gene flow between two distinct shell morphs of the planktonic developing periwinkle *Littorina striata* (Mollusca: Prosobranchia). Marine Ecology Progress Series 163: 155-163.

Dillon RT, Lydeard C (1998) Divergence among moblie basin populations of the pleurocerid snail genus, *Leptoxis*, estimated by allozyme electrophoresis. Malacologia 39: 113-121.

Distel DL (2000) Phylogenetic relationships among Mytilidae (Bivalvia): 18S rRNA data suggest convergence in *Mytilid* body plans. Molecular Phylogenetics and Evolution 15: 25-33.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Duffy JE (1993) Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. Marine Biology 116: 459-470.

Duncombe Rae CM (1991) Agulhas retroflection rings in the south Atlantic ocean: an overview. South African Journal of Marine Science 11: 327-344.

Eanes WF, Koehn RK (1978) An analysis of genetic structure in the monarch butterfly, *Danaus plexippus* L. Evolution 32: 784-797.

Ehrlich PR, Raven PH (1969) Differentiation of populations. Science 165: 1228-1231.

Emanuel BP, Bustamante RH, Branch GM, Eekhout S, Odendaal FJ (1992) A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. South African Journal of Marine Science 12: 341-354.

Endler, JA (1977) Geographic variation, speciation and clines. Princeton University Press. Princeton, New Jersey. p246.

Esselman EJ, Jianqiang L, Crawford DJ, Windus JL, Wolfe AD (1999) Clonal diversity in the rare *Calamagrostis porteri spp. insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. Molecular Ecology 8: 443-451.

Fairbairn DJ (1992) The origins of allometry: size and shape polymorphism in the common waterstrider, *Gerris remigis* Say (Heteroptera, Gerridae). Biological Journal of the Linnean Society 45: 167-186.

Falconer DS (1989) Introduction to quantitative genetics. 3rd Edition. Longman Scientific and Technical, Harlow. p 438.

Fulton JE, Otis JS, Guise KS (1992) Discrimination of walleye, *Stizostedion vitreum vitreum* (Mitchill), stocks by isozyme ananlysis with cellulose acetate. Animal Genetics 23: 221-230.

Gaines SD, Bertness MD (1992) Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360: 579-580.

Gaines MS, Caldwell J, Vivas AM (1974) Genetic variation in the mangrove periwinkle *Littorina angulifera*. Marine Biology 27: 327-332.

Gallardo MH, Carrasco JI (1996) Genetic cohesiveness among populations of *Concholepas concholepas* (Gastropoda, Muricidae) in Southern Chile. Journal of Experimental Marine Biology and Ecology 197: 237-249.

Gallardo MH, Penaloza L, Clasing E (1998) Gene flow and allozymic population structure in the clam *Venus antiqua* (King of Broderip), (Bivalvia, Veneridae) from Southern Chile. Journal of Experimental Marine Biology and Ecology 230: 193-205.

Gibson GD, Chia F-S (1991) Contrasting reporductive modes in two sympatric species of *Haminaea* (Opisthobranchia: Cephalaspidea). Journal of Molluscan Studies 57: 49-60.

Gorman GC, Renzi J (1979) Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 2: 242-249.

Grahame J, Branch GM (1985) Reproductive patterns of marine invertebrates. Oceanography and Marine Biology: an Annual Review 23: 373-398.

Grahame J, Mill PJ (1989) Shell shape variation in *Littorina saxatilis* and *L. arcana*: a case of character displacement. Journal of the Marine Biological Association of the United Kingdom 69: 837-855.

Grahame J, Mill PJ (1992) Local and regional variation in shell shape of rough periwinkles in southern Britain. In: Proceedings of the Third International Symposium on Littorinid Biology. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p99-106.

Grahame J, Mill PJ, Double M, Hull SL (1992) Patterns of variation in AAT-1 allele frequencies in rough periwinkles (*Littorina*) suggest similar selection regimes rather than conspecificity. Journal of the Marine Biological Association of the United Kingdom 72: 499-502.

Grant WS (1988) Protein electrophoresis in population genetics. Mimeographed report, Johannesburg.

Grant WS (1993) Population genetics in southern Africa: what are the questions? South African Journal of Science 89: 113-118.

Grant WS, da Silva-Tatley FM (1997) Lack of genetically-subdivided population structure in *Bullis digitalis*, a southern African marine gastropod with lecithotrophic development. Marine Biology 129: 123-137.

Grant WS, Lang M (1991) Mode of larval development and genetic population structure in *Nodilittorina africana knysnaensis* (Prosobranchia: Littorinidae). Marine Biology 109: 479-483.

Grant WS, Schneider AC, Leslie RW, Cherry MI (1992) Population genetics of the brown mussel *Perna perna* in southern Africa. Journal of Experimental Marine Biology and Ecology 165: 45-58.

Grant WS, Utter FM (1987) Genetic heterogeneity on different geographic scales in *Nucella lamellosa* (Prosobranchia, Thaididae). Malacologia 28: 275-287.

Grassle JF, Grassle JP (1978) Life histories and genetic variation in marine invertebrates. In: Marine Organisms. 1st ed. (Eds: Battaglia B, Beardmore JA) Plenum Press, New York. p347-364.

Guerin G, Bertand M, Billoud B, Meriaux JC (1993) A genetic analysis of variable number of tandem repeats (VNTR) polymorphism in the horse. Genetics Sci Evol 25: 435-445.

Guries RP, Ledig FT (1982) Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill). Evolution 36: 387-402.

Hadrys H, Balick M, Schierwater B (1992) Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology 1: 55-63.

Halbrendt JM, Brown DFJ (1994) Inter- and intraspecific variation in wild-type and single female-derived populations of *Xiphinema americanum*-group Nematodes. Journal of Nematology 26: 212-221.

Hare JA, Cowen RK, Zehr JP, Juanes F, Day KH (1994) Biological and oceanographic insights from larval labrid (Pisces: Labridae) identification using mtDNA sequences. Marine Biology 118: 17-24.

Hare MP, Avise JC (1996) Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). Evolution 50: 2305-2315.

Harris H, Hopkinson DA (1976) Handbook of enzyme electrophoresis in human genetics. Elsevier, Amsterdam.

Harris TFW (1978) Review of coastal currents in Southern African waters. South African National Scientific Programmes Report No 30. Graphic Arts Division of the CSIR, Republic of South Africa.

Harrison RG (1991) Molecular changes at speciation. Annual Review of Ecology and Systematics 22: 281-308.

Hartl DL, Clark AG (1989) Principles of population genetics. 2nd Edition. Sinauer Associates Inc., Sunderland, Massachusetts.

Havenhand JN, Thorpe JP, Todd CD (1986) Estimates of biochemical genetic diversity within and between the nudibranch molluscs *Adlaria proxima* (Alder and Hancock) and *Onchidoris muricata* (Muller) (Doridacea: Onchidorididae). Journal of Experimental Marine Biology and Ecology 95: 105-111.

Hedgecock D (1982) Genetic consequences of larval retention: theoretical and methodological aspects. In: Estuarine Comparisons. (Eds: Kennedy VS) Academic Press, New York. p553-569.

Hedgecock D (1986): Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science 39: 550-564.

Hellberg ME (1995) Stepping-stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and nonequilibrium at different spatial scales. Marine Biology 123: 573-581.

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50: 1167-1175.

Hellberg ME (1998) Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. Evolution 52: 1311-1324.

Herbert PDN, Beaton, MJ (1989) Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, Texas, USA.

Hettle SJH, Hall W, Dyas SL, Beaumont DJ, Caplan DM, Curtis DJ (1997) Assessment of the random amplified polymorphic DNA (RAPD) technique for the analysis of spider populations. The Journal of Arachnology 25: 222-227.

Hilbish TJ, Carson EW, Plante JR, Weaver LA, Gilg MR (2002) Distribution of *Mytilus edulis*, *M. galloprovincialis*, and their hybrids in open-coast populations of mussels in southwestern England. Marine Biology 140: 137-142.

Hillis DM (1984) Misuse and modification of Nei's genetic distance. Systematic Zoology 33: 238-240.

Hillis DM, Moritz C (1990) Molecular Systematics. Sinauer Associates Inc., Sunderland. p587.

Hobday A (1995) Body-size variation exhibited by an intertidal limpet: influence of wave exposure, tidal height and migratory behavior. Journal of Experimental Marine Biology and Ecology 189: 29-45.

Hodgson AN (1999) The biology of siphonariid limpets (Gastropoda: Pulmonata). Oceanography and Marine Biology: an Annual Review 37: 245-314.

Hodgson AN, Farquhar MR, De Villiers CJ (1993) Variations in infestations of *Siphonaria capensis* and *S. concinna* by larval trematodes in Southern Africa. Journal of Molluscan Studies 59: 83-93.

Hoeh WR, Stewart DT, Sutherland BW, Zouros E (1996) Multiple origins of gender-associated mitochonrial DNA lineages in bivalves (Mollusca: Bivalvia). Evolution 50: 2276-2286.

Huang H, Dane F, Kubisiak TL (1998) Allozyme and RAPD analysis of the genetic diverstiy and geographic variation in wild populations of the American chestnut (*Fagaceae*). American Journal of Botany 85: 1013-1021.

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Molecular Ecology 2: 131-137.

Hummel H, Sommer A, Bogaards RH, Portner HO (1997) Variation in genetic traits of the lugworm *Arenicola marina*: temperature related expression of mitochondrial allozymes? Marine Ecology Progress Series 159: 189-195.

Hunt A (1993) Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patiriella calcar* and *P. exigua*. Marine Ecology Progress Series 92: 179-186.

Hurst CD, Skibinski DOF (1995) Comparison of allozyme and mitochondrial DNA spatial differentiation in the limpet *Patella vulgata*. Marine Biology 122: 257-263.

Innes DJ, Bates JA (1999) Morphological variation of *Mytilus edulis* and *Mytilus trossulus* in eastern Newfoundland. Marine Biology 133: 691-699.

Jackman PJH, Pelczynska S (1986) Characterization of *Corynebacterium* group JK by whole-cell protein patterns. Journal of General Microbiology 132: 1911-1915.

Jackson JF, Pounds JA (1979) Comments on assessing the dedifferentiating effect of gene flow. Systematic Zoology 28: 78-85.

James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics: panacea or pandora's box? Annual Review of Ecology and Systematics 21: 129-166.

Janson K (1982a) Genetic and environmental effects on the growth rate of *Littorina saxatilis*. Marine Biology 69: 73-78.

Janson K (1982b) Phenotypic differentiation in *Littorina saxatilis* Olivi (Mollusca, Prosobranchia) in a small area on the Swedish west coast. Journal of Molluscan Studies 48: 167-173.

Janson K (1985a) Genetic variation in three species of Caribbean periwinkles. *Littorina angustior*, *L. lineolata* and *L. ziczac* (Gastropoda: Prosobranchia). Bulletin of Marine Science 37: 871-879.

Janson K (1985b): A morphologic and genetic analysis of *Littorina saxatilis* (Prosobranchia) from Venice, and on the problem of *saxatilis-rudis* nomenclature. Biological Journal of the Linnean Society 24: 51-59.

Janson K (1987) Allozyme and shell variation in two marine snails (*Littorina*, Prosobranchia) with different dispersal abilities. Biological Journal of the Linnean Society 30: 245-256.

Janson K, Sundberg P (1983) Multivariate morphometric analysis of two varieties of *Littorina saxatilis* from the Swedish west coast. Marine Biology 74: 49-53.

Janson K, Ward RD (1984) Microgeographic variation in allozyme and shell characters in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). Biological Journal of the Linnean Society 22: 289-307.

Janson K, Ward RD (1985) The taxonomic status of *Littorina tenebrosa* Montagu as assessed by morphological and genetic analyses. Journal of Conchology 32, 9-15.

Johannesson B (1986) Shell morphology of *Littorina saxatilis* Olivi: the relative importance of physical factors and predation. Journal of Experimental Marine Biology and Ecology 102: 183-195.

Johannesson B, Johannesson K (1996) Population differences in behaviour and morphology in the snail *Littorina saxatilis*: phenotypic plasticity of genetic differentiation? Journal of Zoological Society of London 240: 475-493.

Johannesson K (1988) The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? Marine Biology 99: 507-513.

Johannesson K (1992) Genetic variability and large scale differentiation in two species of littorinid gastropods with planktotrophic development, *Littorina littorea* (L.) and *Melarhaphe (Littorina) neritoides* (L.) (Prosobranchia: Littorinacea), with notes on a mass occurence of *M. neritoides* in Sweden. Biological Journal of the Linnean Society 47: 285-299.

Johannesson K, Johannesson B (1989) Differences in allele frequencies of Aat between high- and mid-rocky shore populations of *Littorina saxatilis* (Olivi) suggest selection in this enzyme locus. Genetic Research 54: 7-11.

Johannesson K, Johannesson B (1990) Genetic variation within *Littorina saxatilis* (Olivi) and *Littorina neglecta* Bean: Is *L. neglecta* a good species? Hydrobiologia 193: 89-97.

Johannesson K, Johannesson B, Lundgren U (1995): Strong natural selection causes microscale allozyme variation in a marine snail. Proceedings of the National Academy of Science of the USA 92: 2602-2606.

Johannesson K, Johannesson B, Rolan-Alvarez E (1993) Morphological differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina saxatilis*. Evolution 47: 1770-1787.

Johannesson K, Kautsky N, Tedengren M (1990) Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations II. Genetic variation. Marine Ecology Progress Series 59: 211-219.

Johannesson K, Tatarenkov A (1997) Allozyme variation in a snail (*Littorina saxatilis*) - deconfounding the effects of microhabitat and gene flow. Evolution 51: 402-409.

Johannesson K, Warmoes T (1990) Rapid colonization of Belgian breakwaters by the direct developer, *Littorina saxatilis* (Olivi) (Prosobranchia, Mollusca). Hydrobiologia 193: 99-108.

Johnson MS, Bentley SL, Ford SS, Ladyman MT, Lambert GJ (2001) Effects of a complex archipelago on genetic subdivision of the intertidal limpet *Siphonaria kurracheensis*. Marine Biology 139: 1087-1094.

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Marine Biology 70: 157-164.

Johnson MS, Black R (1984a) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371-1383.

Johnson MS, Black R (1984b) The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria sp*. Marine Biology 79: 295-302.

Johnson MS, Black R (1991) Genetic subdivision of the intertidal snail *Bembicium vittatum* (Gastropoda: Littorinidae) varies with habitat in the Houtman Abrolhos Islands, Western Australia. Heredity 67: 205-213.

Johnson MS, Black R (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata*. Marine Biology 132: 295-303.

Johnson MS, Black R (1999) *Nodilittorina nodosa* (Gray, 1839) is a plastic morphotype of *Nodilittorina australis* (Gray, 1826). Journal of Molluscan Studies 65: 111-119.

Johnson MS, Clarke B, Murray J (1977) Genetic variation and reproductive isolation in *Partula*. Evolution 31: 116-126.

Johnson MS, Holborn K, Black R (1993) Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Marine Biology 117: 91-96.

Johnson RA, Wichern DW (1998) Applied Multivariate Statistical Analysis. 4th Edition. Prentice Hall, New Jersey. p816.

Johnson SG (2000) Population structure, parasitism, and survivorship of sexual and autodiploid parthenogenetic *Campeloma limum*. Evolution 54: 167-175.

Johnson SG, Bragg E (1999) Age and polyphyletic origins of hybrid and spontaneous parthenogenetic *Campeloma* (Gastropoda: Viviparidae) from the southeastern United States. Evolution 53: 1769-1781.

Jollivet D, Desbruyeres D, Bonhomme F, Moraga D (1995) Genetic differentiation of deep-sea hydrothermal vent alvinellid populations (Annelida: Polychaeta) along the East Pacific Rise. Heredity 74: 376-391.

Jones CS, Okamura B, Noble LR (1994) Parent and larval RAPD fingerprints reveal outcrossing in freshwater bryozoans. Molecular Ecology 3: 193-199.

Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. Nature 804: 802-804.

Jurss K, Rohner M, Bastrop R (1999) Enzyme activities and allozyme polymorphism in two genetic types (or sibling species) of the genus *Marenzelleria* (Polychaeta: Spionidae). Marine Biology 135: 489-496.

Kambhampati S, Black WC, Karamjit SR (1992) Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis and applications. Journal of Medical Entomology 29: 939-945.

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256: 100-102.

Kersters K, De Ley J (1975) Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. Journal of General Microbiology 87: 333-342.

King TL, Eackles MS, Gjetvaj B, Hoeh WR (1999): Intraspecific phylogeography of *Lasmigona subviridis* (Bivalva: Unionidae): conservation implications of range discontinuity. Molecular Ecology 8: 565-578.

Kinsey ST, Orsoy T, Bert TM, Mahmoudi B (1994) Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Marine Biology 118: 309-317.

Klein M, Seitz A (1994) Geographic differentiation between populations of *Rhinocyllus conicus Frolich* (Coleoptera: Curculionidae): concordance of allozyme and morphometric analysis. Zoological Journal of the Linnean Society 110: 181-191.

Knight AJ, Ward RD (1991) The genetic relationships of three taxa in the *Littorina saxatilis* species complex (Prosobranchia: Littorinidae). Journal of Molluscan Studies 57: 81-91.

Knowlton N, Keller BD (1986) Larvae which fall far short of their potential: highly localised recruitment in an alpheid shrimp with extended larval development. Bulletin of Marine Science 39: 213-223.

Kojima S, Segawa R, Hayashi I (1997) Genetic differentiation among populations of the Japanese turban shell *Turbo* (*Batillus*) *cornutus* corresponding to warm currents. Marine Ecology Progress Series 150: 149-155.

Koufopanou V, Reid DG, Ridgway SA, Thomas RH (1999) A molecular phylogeny of the patellid limpets (Gastropoda: Patellidae) and its implications for the origins of their antitropical distribution. Molecular Phylogenetics and Evolution 11: 138-156.

Kyle CJ, Boulding EG (2000) Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Marine Biology 137: 835-845.

Langan-Cranford KM, Pearse JS (1995) Breeding experiments confirm species status of two morphologically similar gastropods (*Lacuna* spp) in central California. Journal of Experimental Marine Biology and Ecology 186: 17-31.

Largier JL, Chapman P, Peterson WT, Swart VP (1992) The western Agulhas bank: circulation, stratigication and ecology. South African Journal of Marine Science 12: 319-339.

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Marine Biology 112: 517-523.

Lessios HA, Kessing BD, Robertson DR (1998) Massive gene flow across the world's most potent marine biogeographic barrier. Proceedings of the Royal Society of London Biological Sciences 265: 583-588.

Lessios HA, Kessing BD, Robertson DR, Paulay G (1999) Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. Evolution 53: 806-817.

Levinton JS, Suchanek TH (1978) Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. Marine Biology 49: 363-375.

Liu H-P, Mitton JB, Wu S-K (1996) Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis*. Evolution 50: 952-957.

Liu LL, Foltz DW, Stickle WB (1991) Genetic population structure of the southern oyster drill *Stramonita* (=*Thais*) *haemostoma*. Marine Biology 111: 71-79.

Liu Z, Furnier GR (1993) Comparison of allozyme, RFLP and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied Genetics 87: 97-105.

Lombard AT, Grant WS (1986) Biochemical population genetics of the black mussel *Choromytilus meridionalis*. South African Journal of Zoology 21: 131-135.

Lowell RB (1984) Desiccation of intertidal limpets: effects of shell size, fit to substratum, and shape. Journal of Experimental Marine Biology and Ecology 77: 197-207.

Lutjeharms JRE, Gordon AL (1987) Shedding of an Agulhas ring observed at sea. Nature 325: 138-140.

Lutjeharms JRE, Valentine HR (1988) Eddies at the subtropical convergence South of Africa. Journal of Physical Oceanography 18: 761-774.

Lydeard C, Holznagel WE, Schnare MN, Gutell RR (2000) Phylogenetic analysis of molluscan mitochondrial LSU rDNA sequences and secondary structures. Molecular Phylogenetics and Evolution 15: 83-102.

Lynch M (1990) The similarity index and DNA fingerprinting. Molecular Biology and Evolution 7: 478-484.

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91-99.

Macleod JAA, Thorpe JP, Duggan NA (1985) A biochemical genetic study of population structure in queen scallop (*Chlamys opercularis*) stocks in the Northern Irish Sea. Marine Biology 87: 77-82.

Mallet AL, Zouros E, Gartner-Kepkay KE, Freeman KR, Dickie LM (1985) Larval viability and heterozygote deficiency in populations of marine bivalves: evidence from pair matings of mussels. Marine Biology 87: 165-172.

Maltagliati F, Camilli L, Biagi F, Abbiati M (1998) Genetic structure of Norway lobster, *Nephrops norvegicus* (L.) (Crustacea: Nephropidae), from the Mediterranean Sea. Scientia Marina 62: 91-99.

Marcus NH (1977) Genetic variation within and between geographically separated populations of the sea urchin, *Arbacia punctulata*. Biological Bulletin 153: 560-576.

Marshall DJ, McQuaid CD (1989) The influence of respiratory responses on the tolerance to sand inundation of the limpets *Patella granularis* L. (Prosobranchia) and *Siphonaria capensis* Q. et G. (Pulmonata). Journal of Experimental Marine Biology and Ecology 128: 191-201.

Marshall DJ, McQuaid CD (1991) Metabolic rate depression in a marine pulmonate snail: pre-adaptation for a terrestrial existence? Oecologia 88: 274-276.

Martel A, Chia F-S (1991) Drifting and dispersal of small bivalves and gastropods with direct development. Journal of Experimental Marine Biology and Ecology 150: 131-147.

McEwan NR, Bakht J, Cecchini E, Coultart A, Geri C, McDonald FM, McDonald MS, Norulaini N, Rahman A, Williamson G (1998) Examination of the pipetting variation between individuals performing random amplified polymorphic DNA (RAPD) analysis. Journal of Microbiological Methods 32: 213-215.

McMahon RF (1992) Microgeographic variation in the shell morphometrics of *Nodlittorina unifasciata* from southwestern Australia in relation to wave exposure of shore. In: Proceedings of the Third International Symposium on Littorinid Biology. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p107-117.

McMillan WO, Raff RA, Palumbi SR (1992) Population genetic consequences of developmental evolution in sea urchins (Genus *Heliocidaris*). Evolution 46: 1299-1312.

McQuaid CD (1992) Stress on the high shore: a review of age-dependent causes of mortality in *Nodilittorina knysnaensis* and *N. africana*. In: Proceedings of the Third International Symposium on Littorinid Biology. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p85-89.

McQuaid CD (1996) Biology of the gastropod family Littorinidae. I. Evolutionay aspects. Oceanography and Marine Biology: an Annual Review 34: 233-262.

McQuaid CD, Cretchley R, Rayner JL (1999) Chemical defence of the intertidal pulmonate limpet *Siphonaria capensis* (Quoy & Gaimard) against natural predators. Journal of Experimental Marine Biology and Ecology 237: 141-154.

Medina M, Walsh PJ (2000) Molecular systematics of the Order Anaspidea based on mitochondrial DNA sequence (12S, 16S, and COI). Molecular Phylogenetics and Evolution 15: 41-58.

Megnegneau B, Debets F, Hoekstra RF (1993) Genetic variability and relatedness in the complex group of black *Aspergilli* based on random amplification of polymorphic DNA. Current Genetics 23: 323-329.

Merritt TJS, Shi L, Chase MC, Rex MA, Etter RJ, Quattro JM (1998) Universal cytochrome *b* primers facilitate intraspecific studies in molluscan taxa. Molecular Marine Biology and Biotechnology 7: 7-11.

Meunier J-R, Grimont PAD (1993) Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Research in Microbiology 144: 373-379.

Michaux JR, Filippucci M-G, Libois RM, Fons R, Matagne RF (1996) Biogeography and taxonomy of *Apodemus sylvaticus* (the woodmouse) in the Tyrrhenian region: enzymatic variations and mitochondrial DNA restriction pattern analysis. Heredity 76: 267-277.

Micheli MR, Bova R, Pascale E, D'Ambrosio E (1994) Reproducible DNA fingerprinting with the random amplified polymorphic DNA (RAPD) method. Nucleic Acids Research 22: 1921-1922.

Mileikovsky SA (1971) Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Marine Biology 10: 193-313.

Mill PJ, Grahame J, Reid DG (1992) Clinal changes in esterase variability in *Littorina saxatilis* (Olivi) and *L. arcana* Hannaford Ellis in southern Britain. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p31-37.

Miller KJ (1998) Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. Marine Ecology Progress Series 163: 225-233.

Mitton JB (1994) Molecular approaches to population biology. Annual Review of Ecology and Systematics 25: 45-69.

Mitton JB, Berg Jr CJ, Orr KS (1989) Population structure, larval dispersal and gene flow in the queen conch, *Strombus gigas*, of the Caribbean. Biological Bulletin 177: 356-362.

Moore J, Ali R (1984) Are dispersal and inbreeding avoidance related? Animal Behaviour 32: 94-112.

Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. Trends in Ecology & Evolution 14: 389-393.

Mukaratirwa S, Kristensen TK, Siegismund HR, Chandiwana SK (1998) Genetic and morphological variation of populations belonging to the *Bulinus truncatus/tropicus* complex (Gastropoda: Planorbidae) in south western Zimbabwe. Journal of Molluscan Studies 64: 435-446.

Murray-Jones SE, Ayre DJ (1997) High levels of gene flow in the surf bivalve *Donax deltoides* (Bivalvia: Donacidae) on the east coast of Australia. Marine Biology 128: 83-89.

Nei M (1972) Genetic distance between populations. American Naturalist 106: 283-292.

Nei M (1978) Estimation of average heterozygosity and genetic distance from small number of individuals. Genetics 89: 583-590.

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. Annual Review of Ecology and Systematics 28: 105-128.

Nevo E, Bieles A, Ben-Shlomo R (1984) The evolutionary significance of genetic diversity: ecological, demographic and life-history correlates. Evolutionary dynamics of genetic diversity. Lecture Notes in Biomathematics 53: 13-213.

Newkirk GF, Doyle RW (1975) Genetic analysis of shell-shape variation in *Littorina saxatilis* on an environmental cline. Marine Biology 30: 227-237.

Newkirk GF, Doyle RW (1979) Clinal variation at an esterase locus in *Littorina saxatilis* and *L. obstusata*. Canadian Journal of Genetics and Cytology 21: 505-513.

Njiokou F, Delay B, Bellec C, N'Goran EK, Yapi Yapi G, Jarnes P (1994) Population genetic structure of the schistosome-vector snail *Bulinus globosus*: examining the role of genetic drift, migration and human activities. Heredity 72: 488-497.

Noy R, Lavie B, Nevo E (1987) The niche-width variation hypothesis revisited: genetic diversity in the marine gastropods *Littorina punctata* (Gmelin) and *L. neritoides* (L.). Journal of Experimental Marine Biology and Ecology 109: 109-116.

Nxomani CD, Ribbink AJ, Kirby R (1994) Differentiation of isolated, threatened fish populations in dolomitic waters of the Transvaal, South Africa, by polyarcylamide gel electrophoresis (PAGE) of total cellular proteins. Biological Conservation 69: 185-189.

Oakey HJ, Gibson LF, George AM (1998) Co-migration of RAPD-PCR amplicons from *Aeromonas hydrophila*. FEMS Microbiology Letters 164: 35-38.

O'Foighil D (1989) Planktotrophic larval development is associated with a restricted geographic range in *Lasaea*, a genus of brooding, hermaphroditic bivalves. Marine Biology 103: 349-358.

Oliverio M (1995) Larval development and allozyme variation in the East Atlantic *Columbella* (Gastropoda: Prosobranchia: Columbellidae). Scientia Marina 59: 77-86.

Olson RR (1985) The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology 66: 30-39.

Ortega S (1985) Competitive interactions among tropical intertidal limpets. Journal of Experimental Marine Biology and Ecology 90: 11-25.

Ostellari ZL, Maccatrozzo L, Bargelloni ML, Battaglia B, Patamello T (1998) Molecular evidence for genetic subdivision of Antarctic krill (*Euphausia superba* DANA) populations. Nature 325: 31-36.

Palmer AR (1990) Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). Hydrobiologia 193: 155-182.

Palumbi SR (1995) Using genetics as an indirect estimator of larval dispersal. In: Ecology of marine invertebrate larvae. (Ed: McEdward L) CRC Press, Boca Raton. p369-389.

Peakall R, Smouse PE, Huff DR (1995) Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss, *Buchloe dactyloides*.Molecular Ecology 4: 135-147.

Pechenik JA (1979) Role of encapsulation in invertebrate life histories. American Naturalist 114: 859-870.

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series 177: 269-297.

Perron FE, Kohn AJ (1985) Larval dispersal and geographic distribution in coral reef gastropods of the genus *Conus*. Proceedings of the Fifth International Coral Reef Congress, Tahiti 4:95-100.

Peterson MA (1996) Long-distance gene flow in the sedentary butterfly, *Euphilotes enoptes* (Lepidoptera: Lycaenidae). Evolution 50: 1990-1999.

Petit RJ, Bahrman N, Baradat PH (1995) Comparison of genetic differentiation in maritime pine (*Pinus pinaster* Ait.) estimated using isozyme, total protein and terpanic loci. Heredity 75: 382-389.

Plomion C, Bahrman N, Durel C-E, O'Malley DM (1995) Genomic mapping in *Pinus pinaster* (maritime pine) using RAPD and protein markers. Heredity 74: 661-668.

Prince JD, Sellers TL, Ford WB, Talbot SR (1987) Experimental evidence for limited dispersal of haliotid larvae (genus *Haliotis*; Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 106: 243-263.

Puterka GJ, Black WC, Steiner WM, Burton RL (1993) Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. Heredity 70: 604-618.

Quesada H, Skibinski DAG, Skibinski DOF (1996) Sex-biased heteroplasmy and mitochondrial DNA inheritance in the mussel *Mytilus galloprovincialis* Lmk. Current Genetics 29: 423-426.

Quesada H, Zapata C, Alvarez G (1995) A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. Marine Ecology Progress Series 116: 99-115.

Raffaelli D (1982) Recent ecological research on some European species of *Littorina*. Journal of Molluscan Studies 48: 342-354.

Rawson PD, Agrawal V, Hilbish TJ (1999) Hybridization between the blue *mussels Mytilus galloprovincialis* and *M. trossulus* along the Pacific coast of North America: evidence for limited introgression. Marine Biology 134: 201-211.

Read GHL (1983) Possible influence of high salinity and low temperature on the distribution of *Macrobrachium petersi* (Hilgendorf) (Crustacea: Caridea) along the south-east coast of South Africa. Transactions of the Royal Society of Southern Africa 45: 35-43.

Reid DG, Rumbak E, Thomas RH (1996) DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. Philosophical Transactions of the Royal Society of London Series B –Biological Sciences 351: 877-895.

Reimchen TE (1982) Shell size divergence in *Littorina mariae* and *L. obtusata* and predation by crabs. Canadian Journal of Zoology 60: 687-695.

Ridgway TM, Branch GM, Stewart BA, Hodgson AN (2000) Taxonomic status of the "*Patella miniata*" species complex (Mollusca: Gastropoda) in southern Africa. Hydrobiologia 420: 103-118.

Ridgway TM, Stewart BA, Branch GM (1999) Limited population differentiation in the bearded limpet *Patella barbara* (Gastropoda: Patellidae) along the coast of South Africa. Journal of the Marine Biological Association of the United Kingdom 79: 639-651.

Ridgway TM, Stewart BA, Branch GM, Hodgson AN (1998) Morphological and genetic differentiation of *Patella granularis* (Gastropoda: Patelliidae): recognition of two sibling species along the coast of southern Africa. Journal of the Zoological Society of London 245: 317-333.

Rieseberg LH (1996) Homology among RAPD fragments in interspecific comparisons. Molecular Ecology 5: 99-105.

Roe KJ, Lydeard C (1998) Molecular systematics of the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). Malacologia 39: 195-205.

Ross TK (1999) Phylogeography and conservation genetics of the Iowa Pleistocene snail. Molecular Ecology 8: 1363-1373.

Ruckelshaus MH (1998) Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina*. Evolution 52: 330-343.

Rus-Kortekaas W, Smulders MJM, Arens P, Vosman B (1994) Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome 37: 375-381.

Russo CAM, Sole-Cava AM, Thrope JP (1994) Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actinidae) with different reproductive strategies. Marine Biology 119: 267-276.

Schaeffer SW, Keller EC, Buroker NE (1985) Population genetics of *Melampus bidentatus* (Gastropoda: Pulmonata): the effect of planktonic development on gene flow. Genetica 66: 223-229.

Scheltema RS (1971) Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. Biological Bulletin 140: 284-322.

Scheltema RS (1975) Relationship of larval dispersal, gene flow and natural selection to geographic variation of benthic invertebrates in estuaries and along coastal regions. Estuarine Research 1: 372-391.

Schierwater B, Ender A (1993) Different thermostable DNA polymerases may amplify different RAPD products. Nucleic Acids Research 21: 4647-4648.

Schizas NV, Street GT, Coull BC, Chandler GT, Quattro JM (1999) Molecular population structure of the marine benthic copepod *Microarthridion littorale* along the southeastern and Gulf coasts of the USA. Marine Biology 135: 399-405.

Schlotterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetacean species. Nature 354: 63-65.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) Arlequin ver. 1.1: A software program for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schumann EH, Perrins L-A, Hunter IT (1982) Upwelling along the south coast of the Cape Province, South Africa. South African Journal of Science 78: 238-242.

Schumann EH, Ross GJB, Goschen WS (1988) Cold water events in Algoa Bay and along the Cape south coast, South Africa, in March/April 1987. South African Journal of Science 84: 579-583.

Scribner KT, Arntzen JW, Burke T (1994) Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite, and multilocus minisatellite data. Molecular Biology and Evolution 11: 737-748.

Shepherd SA, Lowe D, Partington D (1992) Studies on southern Australian abalone (Genus *Haliotis*) XIII: larval dispersal and recruitment. Journal of Experimental Marine Biology and Ecology 164: 247-260.

Silbiger RN, Christ SA, Leonard AC, Garg M, Lattier DL, Dawes S, Dimsoski P, McCormick F, Wessendarp T, Gordon DA, Roth AC, Smith MK, Toth GP (1998) Preliminary studies on the population genetics of the central stoneroller (*Campostoma anomalum*) from the Great Miami river basin, Ohio. Environmental Monitoring and Assessment 51: 481-495.

Skelton P (1993) Evolution. A biological and palaeontological approach. Addison-Wesley Publishing Company. Wokingham, England.

Skibinski DOF, Beardmore JA, Cross TF (1983) Aspects of the population genetics of *Mytilus* (Mytilidae; Mollusca). Biological Journal of the Linnean Society 19: 137-183.

Skibinski DOF, Gallagher C, Beynon CM (1994) Mitochondrial DNA inheritance. Nature 368: 817-818.

Slatkin M (1981) Estimating levels of gene flow in natural populations. Genetics 99: 323-335.

Slatkin M (1985) Gene flow in natural populations. Annual Review of Ecology and Systematics 16: 393-430.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236: 787-792.

Smith JE (1981) The natural history and taxonomy of shell variation in the periwinkles *Littorina saxatilis* and *Littorina rudis*. Journal of the Marine Biological Association of the United Kingdom 61: 215-241.

Snyder TP, Gooch JL (1973) Genetic differentiation in *Littorina saxatilis* (Gastropoda). Marine Biology 22: 177-182.

Soares AG, Scapini F, Brown AC, McLachlan A (1999) Phenotypic plasticity, genetic similarity and evolutionary inertia in changing environments. Journal of Molluscan Studies 65: 136-139.

Sokal RR, Rohlf FJ (1981) Biometry. 2nd Edition. WH Freeman and Company, San Francisco.

Spencer PBS, Adams M, Marsh H, Miller D, Eldridge MDB (1997) High levels of genetic variability in an isolated colony of rock-wallabies (*Petrogale assimilis*): evidence from three classes of molecular markers. Australian Journal of Zoology 45: 199-210.

Stammers M, Harris J, Evans GM, Hayward MD, Forster JW (1995) Use of random PCR (RAPD) technology to analyse phylogenetic relationships in the *Lolium/Festuca* complex. Heredity 74: 19-27.

Stothard JR, Mgeni AF, Alawi KS, Savioli L, Rollinson D (1997) Observations on shell morphology, enzymes and random amplified polymorphic DNA (RAPD) in *Bulinus africanus* group snails (Gastropoda: planorbidae) in Zanzibar. Journal of Molluscan Studies 63: 489-503.

Stothard JR, Rollinson D (1996) An evaluation of random amplified polymorphic DNA (RAPD) for the identification and phylogeny of freshwater snails of the genus *Bulinus* (Gastropoda: Planorbidae). Journal of Molluscan Studies 62: 165-176.

Strathmann RR (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annual Review of Ecology and Systematics 16: 339-361.

Struhsaker JW (1968) Selection mechanisms associated with intraspecific shell variation in *Littorina picta* (Prosobranchia: mesogastropoda). Evolution 22: 459-480.

Sundberg P (1988) Microgeographic variation in shell characters of *Littorina saxatilis* Olivi - a question mainly of size? Biological Journal of the Linnean Society 35: 169-184.

Swart MKJ, Ferguson JWH (1997) Conservation implications of genetic differentiation in southern African populations of black rhinoceros (*Diceros bicornis*). Conservation Biology 11: 79-83.

Swart MJK, Ferguson JWH, du Toit R, Flamand JRB (1994) Substantial genetic variation in southern African black rhinoceros (*Diceros bicornis*). Journal of Heredity 85: 261-266.

Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. Nature 402: 799-802.

Swings J, Kersters K, De Ley J (1976) Numerical analysis of electrophoretic protein patterns of *Zymomonas* strains. Journal of General Microbiology 93: 266-271.

Swofford DL (2000) PAUP 4.0b3a*: Phylogenetic Analysis Using Parsimony. Sinauer, Sunderland MA.

Tablado A, Gappa JL (2000) Morphometric diversity of the pulmonate limpet *Siphonaria lessoni* in different coastal environments. Scientia Marina 65: 33-42.

Tablado A, Lopez Gappa JJ, Magaldi NH (1994) Growth of the pulmonate limpet *Siphonaria lessoni* (Blainville) in a rocky intertidal area affected by sewage pollution. Journal of Experimental Marine Biology and Ecology 175: 211-226.

Takada Y (1992) Multivariate analysis of shell morphology of *Motoacmea spp.* in Amakusa. Venus 51: 157-185.

Tassanakajon A, Pongsomboon S, Rimphanitchayakit V, Jarayabhand P, Boonsaeng V (1997) Random amplified polymorphic DNA (RAPD) markers for determination of genetic variation in wild populations of the black tiger prawn (*Penaeus monodon*) in Thailand. Molecular Marine Biology and Biotechnology 6: 110-115.

Tatarenkov AN (1992) Allozyme variation in *Littorina brevicula* (Philippi) from Peter the Great Bay (Sea of Japan). In: Proceedings of the Third International Symposium on Littorinid Biology. (Eds: Grahame J, Mill PJ, Reid DG) The Malacological Society of London. p25-30.

Tatarenkov AN, Johannesson K (1994) Habitat related allozyme variation on a microgeographic scale in the marine snail *Littorina mariae* (Prosobranchia: Littorinacea). Biological Journal of the Linnean Society 53: 105-125.

Thorpe RS, McGregor DP, Cumming AM, Jordan WC (1994) DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA, RFLP, cytochrome B, Cytochrome oxidase, 12S rRNA sequence and nuclear RAPD analysis. Evolution 48: 230-240.

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews 25: 1-45.

Thuesen EV, Numachi K, Nemoto T (1993) Genetic variation in the planktonic chaetognaths *Parasagitta elegans* and *Eukrohnia hamata*. Marine Ecology Progress Series 101: 243-251.

Todd CD, Havenhand JN, Thorpe JP (1988) Genetic differentiation, pelagic larval transport and gene flow between local populations of the intertidal marine mollusc *Adalaria proxima* (Alder and Hancock). Functional Ecology 2: 441-451.

Todd CD, Lambert WJ, Thorpe JP (1994) The genetic structure of intertidal populations of two species of mollusc on the Scottish west coast: some biogeographic considerations and an assessment of realized larval dispersal. In: The islands of Scotland: a living marine heritage. (Eds: Baxter JM, Usher M) HMSO Publications. p67-87.

Todd CD, Lambert WJ, Thorpe JP (1998) The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotropic larval stages: are pelagic larvae "for" dispersal? Journal of Experimental Marine Biology and Ecology 228: 1-28.

Todd CD, Walker AM, Wolff K, Northcott SJ, Walker AF, Ritchie MG, Hoskins R, Abbott RJ, Hazon N (1997) Genetic differentiation of populations of the copepod sea louse *Lepeophtheirus salmonis* (Kroyer) ectoparasitic on wild and farmed salmonids around the coasts of Scotland: Evidence from RAPD markers. Journal of Experimental Marine Biology and Ecology 210: 251-274.

Trussell GC, Johnson AS, Rudolph SG, Gilfillan ES (1993) Resistance to dislodgement: habitat and size-specific differences in morphology and tenacity in an intertidal snail. Marine Ecology Progress Series 100: 135-144.

Turner TF, Trexler JC, Harris JL, Haynes JL (2000) Nested cladistic analysis indicates population fragmentation shapes genetic diversity in a freshwater mussel. Genetics 154: 777-785.

Urabe M (1998) Contribution of genetic and environmental factors to shell shape variation in the lotic snail *Semisulcospira reiniana* (Prosobranchia: Pleuroceridae). Journal of Molluscan Studies 64: 329-343.

Uthicke S, Benzie JAH (2000) Allozyme electrophoresis indicates high gene flow between populations of *Holothuria (Microthele) nobilis* (Holothuroidea: Aspidochirotida) on the Great Barrier Reef. Marine Biology 137: 819-825.

Uthicke S, Benzie JAH (2001) Restricted gene flow between *Holothuria scabra* (Echinodermata: Holothuroidea) populations along the north-east coast of Australia and the Solomon Islands. Marine Ecology Progress Series 216: 109-117.

Uthicke S, Conand C, Benzie JAH (2001) Population genetics of the fissiparous holothurians *Stichopus chloronotus* and *Holothuria atra* (Aspidochirotida): a comparison between the Torres Strait and La Reunion. Marine Biology 139: 257-265.

Vance RR (1973) On reproductive strategies in marine benthic invertebrates. American Naturalist 107: 339-352.

Vera Cruz CM, Gossele F, Kersters K, Segers P, Van Den Mooter M, Swings J, De Ley J (1984) Differentiation between *Xanthomonas campestris pv. oryzae*, *Xanthomonas campestris pv. oryzicola* and the bacterial "brown Blotch" pathogen on rice by numerical analysis of phenotypic features and protein gel electrophoregrams. Journal of General Microbiology 130: 2983-2999.

Vermeij GJ (1973) Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. Marine Biology 20: 319-346.

Vermeij GJ (1982a) Environmental change and the evolutionary history of the periwinkle (*Littorina littorea*) in North America. Evolution 36: 561-580.

Vermeij GJ (1982b) Phenotypic evolution in a poorly dispersing snail after arrival of a predator. Nature 299: 349-350.

Vermeij GJ (1987) The dispersal barrier in the tropical pacific: implications for Molluscan speciation and extinction. Evolution 41: 1046-1058.

Villet MH, McKitterick L, Robertson HG (1999) Systematic status of *Plectroctena mandibularis* Smith and *P. conjugata* Santschi (Hymenoptera: Formicidae: Ponerini). African Entomology 7: 277-284.

von Soosten C, Schmidt H, Westheide W (1998) Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma* (Polychaeta: Syllidae). Results from RAPD-PCR investigations. Marine Biology 131: 659-669.

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution 42: 995-1005.

Ward RD (1990) Biochemical genetic variation in the genus *Littorina* (Prosobranchia: Mollusca). Hydrobiologia 193: 53-69.

Ward RD, Janson K (1985) A genetic analysis of sympatric subpopulations of the sibling species *Littorina saxatilis* (Olivi) and *Littorina arcana* Hannaford Ellis. Journal of Molluscan Studies 51: 86-94.

Ward RD, Warwick T (1980) Genetic differentiation in the molluscan species *Littorina rudis* and *Littorina arcana* (Prosobranchia: Littorinidae). Biological Journal of the Linnean Society 14: 417-428.

Ward RD, Warwick T, Knight AJ (1986) Genetic analysis of ten polymorphic enzyme loci in *Littorina saxatilis* (Prosobranchia: Mollusca). Heredity 57: 233-241.

Warnke K, Soller R, Blohm D, Saint-Paul U (2000) Rapid differentiation between *Octopus vulgaris* Cuvier (1797) and *Octopus mimus* Gould (1852), using randomly amplified polymorphic DNA. Journal of Zoological Systematic Evolutionary Research 38: 119-122.

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Marine Biology 105: 145-151.

Weber LI, Gray D, Hodgson AN, Hawkins SJ (1997) Genetic divergence between South African *Helcion* species and north-east Atlantic *H. pellucidum* (Mollusca: Patellogastropoda). Journal of the Marine Biological Association of the United Kingdom 77:1139-1150.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wilding CS, Beaumont AR, Latchford JW (1997) Mitochondrial DNA variation in the scallop Pecten maximus (L.) assessed by a PCR-RFLP method. Heredity 79: 178-189.

Wilding CS, Grahame J, Mill PJ (2000) Mitochondrial DNA CoI haplotype variation in sibling species of rough periwinkles. Heredity 85: 62-74.

Wilding CS, Mill PJ, Grahame J (1999) Partial sequence of the mitochondrial genome of *Littorina saxatilis*: relevance to gastropod phylogenetics. Journal of Molecular Evolution 48: 348-359.

Wilkins NP, O'Regan D (1980) Genetic variation in sympatric sibling species of *Littorina*. The Veliger 22: 355-359.

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology 218: 704-742.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Williams ST, Benzie JAH (1993) Genetic consequences of long larval life in the starfish *Linckia laevigata* (Echinodermata: Asteroidea). Marine Biology 117: 71-77.

Williams ST, Benzie JAH (1996) Genetic uniformity of widely separated populations of the coral reef starfish *Linckia laevigata* from the East Indian and West Pacific Oceans, revealed by allozyme electrophoresis. Marine Biology 126: 99-107.

Wilson IF, Gosling EM, Tapper W (1999) The systematic status of the lagoon periwinkle, *Littorina tenebrosa*. Journal of the Marine Biological Association of the United Kingdom 79: 653-660.

Worcester SE (1994) Adult rafting versus larval swimming: dispersal and recruitment of a botryllid ascidian on eelgrass. Marine Biology 121: 309-317.

Wright S (1978) Evolution and the genetics of populations Vol 4. Variability within and among natural populations. University of Chicago Press, Hawaii, Honolulu.

Wright JT, Zuccarello GC, Steinberg PD (2000) Genetic structure of the subtidal red alga *Delisea pulchra*. Marine Biology 136: 439-448.

Yu J-K, Wang H-Y, Lee S-C, Dai C-F (1999) Genetic structure of a scleractinian coral, *Mycedium elephantotus*, in Taiwan. Marine Biology 133: 21-28.

Appendix I: Formulae used for the analysis of the allele frequency data.

1. Hardy-Weinberg Equilibrium
Departures from Hardy-Weinberg equilibrium were tested using the G test for goodness of fit, following the formula laid out by Sokal and Rohlf (1981):

$G = 2\Sigma^{fi} \ln (f_i/f)$
where f_i is the observed allele frequency and f is the expected allele frequency; with degrees of freedom being $(x^2-x)/x$; where x is the number of allele classes per locus.

2. Gene Diversity (H)
This is also known as expected heterozygosity and is a measure of within population variation. This is calculated from allele frequencies with the assumption that mating is random (Grant, 1994).

$H = 1 - \Sigma^m p_i^2$
where p_i is the allele frequency of a locus and m is the number of alleles.

Average heterozygosity (H) is the mean of individual locus heterozygosities over all loci including the monomorphic loci within a single population;

mean $H = \Sigma H_j/r$
where r is the total number of loci (Grant, 1994).

3. F statistics
These were originally developed by Wright (1978) and are used to describe various aspects of non-random mating within populations. They differ mainly according to their reference populations (Hartl and Clark, 1989). All the statistics are based on an inbreeding coefficient F, which measures deviation from random mating. The following three F coefficients are related to one another by;

$(1-F_{IT}) = (1-F_{IS})(1-F_{ST})$
(Grant, 1994).

F_{IS} is the average inbreeding coefficient over subpopulations and it measures the average deviation from Hardy-Weinberg equilibrium (Grant, 1994). It can also be described as inbreeding in individuals relative to the subpopulation to which they belong (Hartl and Clark, 1989).

$F_{IS} = (h_E - h_O)/h_E$
where h_E is expected heterozygosity and h_O is observed heterozygosity for a locus in a subpopulation. F_{IS} is then mean F over all loci. If F_{IS} has a positive value this indicates inbreeding while a negative value indicates that outbreeding is occurring (Grant, 1994).

F_{IT} is an overall breeding coefficient including a contribution from a division into subpopulations and non-random mating within subpopulations (Grant, 1994). Again, this F value can be alternatively described as inbreeding in individuals relative to the total population (Hartl and Clark, 1989).

F_{ST} is the measure of the amount of allele frequency differentiation among subpopulations due

to random drift. It is also defined as the ratio of observed variance of allele frequency to the maximum theoretical variance for a given allelic frequency.

$F_{ST} = \sigma_p^2/(p[1-p])$
where p is mean allelic frequency among populations and σ is variance in frequency of either allele (Grant,1994).

In this study, however, an unbiased estimate of F_{ST} was used so that the contribution of each allele at each locus was averaged and the variance components then summed separately. This avoids heavy weighting of either high or low frequency alleles within the original data set. Thus F_{ST} was calculated as:

$F_{ST} = \Sigma^r \Sigma^m (\sigma p_{ij}^2/p_{ij}[1-p_{ij}]/\Sigma m_i-1)$
where i is the variance of the alleles, j is the variance of the loci, r is the total number of loci and m is the number of alleles (Wright, 1978).

4. Gene flow (Nm)
Nm is the product of effective population size (N) multiplied by the proportion of individuals migrating between populations in one generation (m). As a result Nm is defined as an estimate of the number of individuals exchanged between populations in a single generation and is calculated as:

$Nm = (1/F_{ST}-1)/4$ (Crow and Kimura, 1970)

As a general rule of thumb Nm values greater than 1 indicate substantial gene flow between populations (Slatkin, 1987).

5. Nei's genetic distance (D)
Nei's original genetic distance value was calculated as:

$D = -\log_e I$
where $I_j = \Sigma x_i y_j/\sqrt{\Sigma x_i^2 \Sigma y_j^2}$
where in turn x_i and y_j are frequencies of the ith allele at the jth locus in populations X and Y (Nei, 1972). Later, Hillis (1984) proposed a modification to the calculation of D, so that D itself was not "adversely affected" by changing rates of variation at different loci among populations. Therefore, genetic identity (I) becomes calculated as:
$I^* = \Sigma I_j/L$
where L is the number of loci
Then genetic distance (D) becomes:
$D^* = -\log_e I^*$
Using this calculation D^* is then not distorted by shared or unshared polymorphism as was the case previously when Nei's original assumptions were not met. Also D^* is not distorted by small I_j values (Hillis, 1984).

Appendix II: Allele frequencies for 5 polymorphic loci in *S. capensis*.
(p(A or B) = proportions of allele A or B; n = sample size)

	Cape coast											Transkei coast												
Enzyme	1	2	3	5	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29
AAT																								
p(A)	1.0000	1.0000	1.0000	1.0000	0.6250	0.8750	0.8750	0.8750	1.0000	0.9167	1.0000	0.7500	0.8636	1.0000	0.6111	0.4545	0.8333	0.7500	0.7941	0.6818	0.7083	0.9091	1.0000	0.7917
p(B)	0.0000	0.0000	0.0000	0.0000	0.3750	0.1250	0.1250	0.1250	0.0000	0.0833	0.0000	0.2500	0.1364	0.0000	0.3889	0.5455	0.1667	0.2500	0.2059	0.3182	0.2917	0.0909	0.0000	0.2083
n	12	11	12	12	12	12	12	12	12	12	12	12	11	11	9	11	12	12	17	11	12	11	12	12
ACP																								
p(A)	0.7500	0.7917	0.9583	1.0000	0.9167	0.7917	0.3333	1.0000	1.0000	1.0000	0.8333	0.7083	0.9167	1.0000	0.7500	0.5833	0.7917	0.9167	1.0000	0.8333	0.5833	0.7917	0.7083	1.0000
p(B)	0.2500	0.2083	0.0417	0.0000	0.0833	0.2083	0.6667	0.0000	0.0000	0.0000	0.1667	0.2917	0.0833	0.0000	0.2500	0.4167	0.2083	0.0833	0.0000	0.1667	0.4167	0.2083	0.2917	0.0000
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	17	12	12	12	12	12
MDH																								
p(A)	1.0000	0.6667	1.0000	0.9167	0.9583	0.7500	0.5909	0.8333	0.9091	0.9167	0.6667	0.9583	0.6667	1.0000	0.9167	1.0000	0.9583	0.7727	0.7500	1.0000	0.9091	0.8333	0.9167	0.9583
p(B)	0.0000	0.3333	0.0000	0.0833	0.0417	0.2500	0.4091	0.1667	0.0909	0.0833	0.3333	0.0417	0.3333	0.0000	0.0833	0.0000	0.0417	0.2273	0.2500	0.0000	0.0909	0.1667	0.0833	0.0417
n	12	12	12	12	12	12	11	12	11	12	12	12	12	12	12	12	12	11	18	12	11	12	12	12
MPI																								
p(A)	0.0000	0.1667	0.2917	0.0833	0.0000	0.0000	0.0417	0.3750	0.0417	0.5000	0.1250	0.1250	0.2917	0.0000	0.0000	0.1250	0.0000	0.5455	0.4583	0.1250	0.5000	0.7727	0.2727	0.0833
p(B)	1.0000	0.8333	0.7083	0.9167	1.0000	1.0000	0.9583	0.6250	0.9583	0.5000	0.8750	0.8750	0.7083	1.0000	1.0000	0.8750	1.0000	0.4545	0.5417	0.8750	0.5000	0.2273	0.7273	0.9167
n	12	12	12	12	12	12	12	12	12	12	12	12	12	11	12	12	12	11	12	12	12	11	11	12
PGM																								
p(A)	0.8750	0.9167	1.0000	0.7083	0.7917	1.0000	0.6364	1.0000	1.0000	0.1667	0.3333	0.4583	1.0000	0.3750	0.1000	0.1667	0.0000	0.9167	0.7500	0.5500	0.1500	0.4500	0.0000	0.7917
p(B)	0.1250	0.0833	0.0000	0.2917	0.2083	0.0000	0.3636	0.0000	0.0000	0.8333	0.6667	0.5417	0.0000	0.6250	0.9000	0.8333	1.0000	0.0833	0.2500	0.4500	0.8500	0.5500	1.0000	0.2083
n	12	12	12	12	12	12	11	12	11	9	12	12	12	12	10	12	12	12	12	10	10	10	9	12

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg, 17 East London; 18 Cintsa; 19Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 25 Hluleka; 26 Umngazi; 27 Port St John's; 28 Mkambati; 29 Port Edward

Appendix III: Values of gene diversity (H) for polymorphic loci in *S. capensis*

	Cape coast											Transkei coast												
Enzyme	1	2	3	5	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29
AAT																								
H	0.0000	0.0000	0.0000	0.0000	0.4891	0.2283	0.2283	0.2283	0.0000	0.1594	0.0000	0.3913	0.2468	0.0000	0.5033	0.5195	0.2899	0.3913	0.3369	0.4545	0.4312	0.1732	0.0000	0.3442
G	n/a	n/a	n/a	n/a	0.0170	0.0233	0.0233	0.0233	n/a	0.0021	n/a	0.0106	0.0215	n/a	0.2979	0.3088	0.0215	0.0965	0.0782	0.0272	0.0383	0.0026	n/a	0.1769
ACP																								
H	0.3913	0.3442	0.0833	0.0000	0.1594	0.3442	0.4638	0.0000	0.0000	0.0000	0.2899	0.4312	0.1594	0.0000	0.3913	0.5072	0.3442	0.1594	0.0000	0.2899	0.5072	0.3442	0.4312	0.0000
G	0.0105	0.0001	0.0002	n/a	0.0575	0.1769	0.7883	n/a	n/a	n/a	0.0055	0.0001	0.0021	n/a	0.0965	0.0317	0.1769	0.0575	n/a	0.0055	0.3565	0.0415	0.0383	n/a
MDH																								
H	0.0000	0.4638	0.0000	0.1594	0.0833	0.3913	0.5065	0.2899	0.1732	0.1594	0.4638	0.0833	0.4638	0.0000	0.1594	0.0000	0.0833	0.3680	0.3857	0.0000	0.1732	0.2899	0.1594	0.0833
G	n/a	0.0047	n/a	0.0021	0.0002	0.0105	0.0185	0.0215	0.0026	0.0021	0.0047	0.0002	0.2278	n/a	0.0575	n/a	0.0002	0.0009	0.0439	n/a	0.0564	0.0215	0.0021	0.0002
MPI																								
H	0.0000	0.2899	0.4312	0.1594	0.0000	0.0000	0.0833	0.4891	0.0833	0.5217	0.2283	0.2283	0.4312	0.0000	0.0000	0.2283	0.0000	0.5195	0.5181	0.2283	0.5217	0.3680	0.4156	0.1594
G	n/a	0.0055	0.3687	0.0575	n/a	n/a	0.0002	0.5583	0.0002	0.4572	0.0233	0.0080	0.0383	n/a	n/a	0.0233	n/a	0.3088	0.7255	0.0233	0.4572	0.1688	0.0854	0.0021
PGM																								
H	0.2283	0.1594	0.0000	0.4312	0.3442	0.0000	0.4848	0.0000	0.0000	0.2941	0.4683	0.5181	0.0000	0.4891	0.1895	0.2899	0.0000	0.1594	0.3913	0.5211	0.2684	0.5211	0.0000	0.3442
G	0.0233	0.0575	n/a	0.3687	0.1769	n/a	0.2024	n/a	n/a	0.0167	0.0047	0.0351	n/a	0.1136	0.0032	0.2873	n/a	0.0575	0.5403	0.4805	0.0193	0.4805	n/a	0.1769
Mean																								
H	0.1167	0.1833	0.0333	0.0500	0.1167	0.1333	0.1606	0.1000	0.0530	0.1222	0.1833	0.3000	0.1348	0.0500	0.0956	0.1197	0.1000	0.1606	0.1520	0.1245	0.1367	0.1912	0.1197	0.0833
G	0.1239	0.2514	0.1029	0.1500	0.2152	0.1928	0.3533	0.2014	0.0513	0.2269	0.2891	0.3304	0.2602	0.0978	0.2487	0.3090	0.1435	0.3195	0.3264	0.2987	0.3803	0.3392	0.2012	0.1862

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg, 17 East London; 18 Cintsa; 19Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 25 Hluleka; 26 Umngazi; 27 Port St John's; 28 Mkambati; 29 Port Edward

Appendix IV: Pairwise F_{ST} values (above the diagonal) and pairwise Nm values (below the diagonal) for single populations of *S. capensis*. ($ = infinite amount of gene flow). Mann Whitney U test for differences between coastal regions; F_{ST}: U = 2093.5; Z = -0.235; p = 0.814; Nm: U = 1829.5; Z = 0.979; p = 0.328.

	Cape coast											Transkei coast												
Popn	1	2	3	5	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29
1	-----	0.10	0.14	0.07	0.10	0.09	0.16	0.16	0.08	0.29	0.20	0.12	0.15	0.30	0.28	0.31	0.25	0.20	0.21	0.13	0.28	0.25	0.28	0.08
2	2.19	-----	0.10	0.09	0.13	0.03	0.11	0.06	0.08	0.25	0.13	0.15	0.02	0.26	0.28	0.31	0.27	0.08	0.10	0.16	0.25	0.19	0.26	0.10
3	1.51	2.36	-----	0.85	0.15	0.14	0.30	0.05	0.07	0.19	0.24	0.18	0.09	0.27	0.32	0.41	0.19	0.09	0.11	0.19	0.28	0.23	0.18	0.07
5	3.31	2.44	2.70	-----	0.07	0.10	0.18	0.11	0.07	0.19	0.11	0.11	0.11	0.06	0.22	0.27	0.19	0.13	0.11	0.09	0.27	0.18	0.24	0.04
9	2.28	1.64	1.48	3.39	-----	0.10	0.18	0.12	0.10	0.23	0.18	0.07	0.13	0.15	0.16	0.16	0.21	0.13	0.12	0.02	0.24	0.22	0.28	0.01
10	2.62	7.28	1.48	2.23	2.36	-----	0.13	0.12	0.07	0.30	0.15	0.15	0.06	0.26	0.26	0.85	0.16	0.10	0.16	0.15	0.28	0.26	0.17	0.09
11	1.35	2.11	0.59	1.12	1.15	1.71	-----	0.26	0.24	0.31	0.11	0.10	0.20	0.23	0.20	0.21	0.24	0.22	0.21	0.16	0.20	0.22	0.24	0.19
12	1.30	3.96	4.83	2.01	1.77	1.88	0.72	-----	0.10	0.21	0.19	0.18	0.02	0.28	0.31	0.34	0.20	0.00	0.05	0.16	0.25	0.18	0.16	0.09
13	2.73	2.87	3.54	3.14	2.36	3.62	0.78	2.30	-----	0.32	0.22	0.20	0.09	0.22	0.29	0.35	0.26	0.13	0.17	0.17	0.37	0.29	0.35	0.07
14	0.63	0.77	1.07	1.10	0.83	0.58	0.56	0.94	0.55	-----	0.10	0.13	0.21	0.14	0.17	0.20	0.15	0.16	0.13	0.14	0.09	0.07	0.08	0.21
15	1.28	1.46	1.62	3.47	1.11	1.38	2.09	1.09	0.91	2.17	-----	0.08	0.15	0.12	0.13	0.18	0.14	0.20	0.15	0.12	0.14	0.13	0.10	0.15
16	1.87	1.39	1.12	2.09	3.47	1.44	2.25	1.11	1.02	1.63	2.76	-----	0.17	0.11	0.06	0.05	0.10	0.17	0.15	0.00	0.08	0.13	0.12	0.08
17	1.46	13.09	2.53	2.04	1.62	3.80	1.03	15.96	2.41	0.97	1.43	1.25	-----	0.25	0.28	0.32	0.19	0.01	0.05	0.15	0.24	0.18	0.16	0.09
18	0.57	0.72	0.66	3.74	1.46	0.73	0.84	1.66	0.92	1.53	1.92	2.04	0.77	-----	0.17	0.25	0.15	0.27	0.23	0.11	0.22	0.22	0.20	0.11
19	0.66	0.66	0.52	0.91	1.28	0.70	1.03	0.57	0.60	1.24	1.69	3.69	0.66	1.22	-----	0.01	0.03	0.27	0.26	0.08	0.09	0.23	0.13	0.17
20	0.56	0.56	0.36	0.67	1.32	0.63	0.94	0.48	1.46	0.98	1.12	5.00	0.53	0.77	20.38	-----	0.08	0.30	0.30	0.08	0.06	0.24	0.13	0.21
21	0.75	0.67	1.10	1.04	0.94	1.33	0.78	0.98	0.73	1.47	1.55	2.15	1.08	1.40	7.72	2.77	-----	0.31	0.29	0.12	0.12	0.25	0.09	0.19
22	1.03	2.74	2.47	1.66	1.73	2.15	0.88	$	1.63	1.34	1.02	1.24	20.22	0.68	0.67	0.58	0.56	-----	0.01	0.15	0.19	0.08	0.26	0.09
23	0.96	2.29	2.08	2.01	1.84	1.31	0.94	5.25	1.21	1.64	1.48	1.45	4.61	0.84	0.71	0.60	0.60	23.26	-----	0.11	0.20	0.09	0.24	0.09
25	1.70	1.30	1.09	2.57	11.28	1.40	1.33	1.31	1.20	1.55	1.83	$	1.41	2.04	3.03	2.76	1.81	1.46	2.07	-----	0.11	0.16	0.16	0.03
26	0.65	0.73	0.64	0.68	0.79	0.65	1.03	0.76	0.43	2.49	1.60	3.04	0.81	0.87	2.46	3.96	1.89	1.08	1.03	2.04	-----	0.06	0.07	0.24
27	0.76	1.08	0.86	1.14	0.87	0.71	0.89	1.12	0.62	3.57	1.68	1.66	1.15	0.88	0.86	0.81	0.76	2.74	2.64	1.32	3.96	-----	0.16	0.20
28	0.64	0.73	1.18	0.81	0.65	1.21	0.81	1.30	0.48	2.80	2.33	1.79	1.36	1.00	1.68	1.75	2.49	0.73	0.78	1.28	3.40	1.34	-----	0.24
29	2.74	2.15	3.20	6.63	23.74	2.41	1.09	2.62	3.32	0.97	1.37	2.71	2.43	2.09	1.20	0.93	1.04	2.40	2.43	7.49	0.77	1.00	0.79	-----

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg, 17 East London; 18 Cintsa; 19Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 25 Hluleka; 26 Umngazi; 27 Port St John's; 28 Mkambati; 29 Port Edward

Appendix V: Allele frequencies for 4 polymorphic loci in *S. serrata*
(p(A or B) = proportions of allele A or B; n = sample size)

	Cape coast												Transkei coast													
Enzyme	1	2	3	5	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29
FUM																										
p(A)	0.1250	0.2273	0.3000	1.0000	0.2917	1.0000	0.0000	1.0000	0.0417	1.0000	1.0000	1.0000	0.9167	0.9583	0.7727	0.7917	0.6667	1.0000	1.0000	0.3333	1.0000	1.0000	0.2917	0.4583	1.0000	0.1818
p(B)	0.8750	0.7727	0.7000	0.0000	0.7083	0.0000	1.0000	0.0000	0.9583	0.0000	0.0000	0.0000	0.0833	0.0417	0.2273	0.2083	0.3333	0.0000	0.0000	0.6667	0.0000	0.0000	0.7083	0.5417	0.0000	0.8182
n	12	11	10	12	12	12	12	4	12	12	12	12	12	12	11	12	12	12	9	12	12	12	12	12	12	11
G6PDH																										
p(A)	1.0000	1.0000	1.0000	0.8750	1.0000	1.0000	0.7083	1.0000	1.0000	1.0000	1.0000	0.9167	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
p(B)	0.0000	0.0000	0.0000	0.1250	0.0000	0.0000	0.2917	0.0000	0.0000	0.0000	0.0000	0.0833	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
MDH																										
p(A)	0.8636	0.7273	0.5500	1.0000	0.4583	0.3500	1.0000	1.0000	1.0000	0.7000	0.8333	1.0000	0.1250	0.5455	1.0000	0.9167	0.9583	0.8333	0.6818	0.5000	0.7917	0.5000	0.8333	0.7917	0.7500	0.7083
p(B)	0.1364	0.2727	0.4500	0.0000	0.5417	0.6500	0.0000	0.0000	0.0000	0.3000	0.1667	0.0000	0.8750	0.4545	0.0000	0.0833	0.0417	0.1667	0.3182	0.5000	0.2083	0.5000	0.1667	0.2083	0.2500	0.2917
n	11	11	10	12	12	10	12	12	12	10	12	12	12	11	12	12	12	12	11	11	12	11	12	12	12	12
NP																										
p(A)	1.0000	1.0000	0.8333	1.0000	0.5417	0.9167	1.0000	0.9583	0.9167	1.0000	1.0000	0.9583	1.0000	1.0000	0.8750	0.9167	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8333	1.0000
p(B)	0.0000	0.0000	0.1667	0.0000	0.4583	0.0833	0.0000	0.0417	0.0833	0.0000	0.0000	0.0417	0.0000	0.0000	0.1250	0.0833	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1667	0.0000
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	11	12	12	12

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 7 Gansbaai; 8 Pearly Beach; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg; 17 East London; 18 Cintsa; 19 Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 24 Coffee Bay; 25 Hluleka; 26 Umngazi; 27 Port St Johns; 28 Mkambati; 29 Port Edward

Appendix VI: Values of gene diversity (H) for polymorphic loci in *S. serrata*

	Cape coast												Transkei coast													
Enzyme	1	2	3	5	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29
FUM																										
H	0.2283	0.3680	0.4421	0.0000	0.4312	0.0000	0.0000	0.0000	0.0833	0.0000	0.0000	0.0000	0.1594	0.0833	0.3680	0.3442	0.4683	0.0000	0.0000	0.4638	0.0000	0.0000	0.4312	0.5181	0.0000	0.3117
G	0.0233	0.0356	0.0721	n/a	0.0383	n/a	n/a	n/a	0.0002	n/a	n/a	n/a	0.0575	0.0002	0.1688	0.1769	0.2278	n/a	n/a	0.7883	n/a	n/a	0.3687	0.1839	n/a	0.2831
G6PDH																										
H	0.0000	0.0000	0.0000	0.2283	0.0000	0.0000	0.4312	0.0000	0.0000	0.0000	0.0000	0.1594	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G	n/a	n/a	n/a	0.0233	n/a	n/a	0.3687	n/a	n/a	n/a	n/a	0.0021	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
MDH																										
H	0.2468	0.4156	0.5211	0.0000	0.5181	0.4789	0.0000	0.0000	0.0000	0.4421	0.2899	0.0000	0.2283	0.5195	0.0000	0.1594	0.0833	0.2899	0.4545	0.5238	0.3442	0.5238	0.2899	0.3442	0.3913	0.4312
G	0.0099	0.2399	2.7749	n/a	0.0489	0.0407	n/a	n/a	n/a	0.0020	0.0215	n/a	0.0233	0.0004	n/a	0.0021	0.0002	0.0215	0.5459	0.6665	0.0001	0.1292	0.0215	0.0001	0.0106	0.4007
NP																										
H	0.0000	0.0000	0.2899	0.0000	0.5181	0.1594	0.0000	0.0833	0.1594	0.0000	0.0000	0.0833	0.0000	0.0000	0.2283	0.1594	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2899	0.0000
G	n/a	n/a	0.2873	n/a	0.1839	0.0575	n/a	0.0002	0.0575	n/a	n/a	0.0002	n/a	n/a	0.0233	0.0021	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.2873	n/a
Mean																										
H	0.0890	0.2045	0.2750	0.0208	0.2708	0.1250	0.0208	0.0208	0.0208	0.1000	0.0833	0.0625	0.0208	0.1572	0.0436	0.1042	0.0625	0.0833	0.1591	0.0227	0.1042	0.0682	0.1042	0.1667	0.0833	0.1458
G	0.1188	0.1959	0.3133	0.0571	0.3668	0.1596	0.1078	0.0208	0.0607	0.1105	0.0725	0.0607	0.0969	0.1507	0.1491	0.1658	0.1368	0.0725	0.1136	0.2469	0.0861	0.1310	0.1803	0.2156	0.1703	0.1857

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 7 Gansbaai; 8 Pearly Beach; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg; 17 East London; 18 Cintsa; 19 Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 24 Coffee Bay; 25 Hluleka; 26 Umngazi; 27 Port St Johns; 28 Mkambati; 29 Port Edward

Appendix VII: Pairwise F_{ST} values (above the diagonal) and pairwise Nm values (below the diagonal) for single populations of *S. serrata*. ($ = infinite amount of gene flow). Mann Whitney U test for differences between coastal regions; F_{ST}: U = 2382.0; Z = 2.069; p = 0.04; Nm: U = 2273.5; Z = -2.165; p = 0.03.

	Cape coast												Transkei coast													
Popn	1	2	3	5	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	-----	0.01	0.10	0.35	0.24	0.43	0.13	0.30	0.04	0.45	0.37	0.26	0.72	0.50	0.24	0.21	0.22	0.44	0.45	0.13	0.43	0.54	0.00	0.09	0.32	0.02
2	22.25	-----	0.05	0.36	0.18	0.33	0.23	0.31	0.12	0.38	0.28	0.27	0.58	0.37	0.25	0.19	0.22	0.38	0.37	0.02	0.38	0.41	0.00	0.03	0.29	0.00
3	2.19	5.19	-----	0.33	0.04	0.25	0.26	0.35	0.21	0.27	0.22	0.31	0.32	0.24	0.26	0.21	0.21	0.32	0.26	0.03	0.30	0.26	0.08	0.07	0.25	0.04
5	0.48	0.44	0.51	-----	0.42	0.24	0.25	0.03	0.34	0.17	0.10	0.00	0.31	0.17	0.10	0.07	0.12	0.10	0.18	0.39	0.12	0.27	0.29	0.25	0.12	0.38
7	0.79	1.17	6.13	0.34	-----	0.31	0.35	0.48	0.31	0.39	0.37	0.40	0.39	0.35	0.35	0.33	0.35	0.45	0.38	0.14	0.43	0.37	0.22	0.20	0.31	0.17
8	0.34	0.51	0.75	0.80	0.55	-----	0.35	0.33	0.54	0.09	0.18	0.23	0.03	0.01	0.28	0.22	0.29	0.18	0.08	0.21	0.15	0.00	0.35	0.27	0.12	0.34
9	1.65	0.86	0.70	0.73	0.46	0.48	-----	0.25	0.08	0.34	0.29	0.19	0.67	0.54	0.35	0.27	0.29	0.29	0.34	0.32	0.30	0.40	0.20	0.28	0.26	0.20
10	0.58	0.56	0.47	8.00	0.27	0.51	0.77	-----	0.47	0.14	0.07	0.02	0.29	0.15	0.01	0.02	0.05	0.07	0.16	0.32	0.09	0.24	0.23	0.18	0.11	0.33
11	6.75	1.87	0.93	0.49	0.56	0.22	2.96	0.29	-----	0.41	0.35	0.33	0.58	0.45	0.35	0.25	0.19	0.36	0.42	0.22	0.38	0.48	0.09	0.17	0.39	0.10
12	0.31	0.40	0.68	1.20	0.38	2.58	0.50	1.51	0.35	-----	0.00	0.11	0.24	0.00	0.17	0.10	0.23	0.00	0.00	0.32	0.00	0.02	0.34	0.26	0.05	0.40
13	0.43	0.64	0.91	2.29	0.43	1.11	0.62	3.42	0.46	56.60	-----	0.06	0.33	0.07	0.07	0.02	0.02	0.00	0.03	0.22	0.00	0.18	0.19	0.06	0.05	0.31
14	0.73	0.68	0.57	$	0.38	0.82	1.06	10.75	0.50	2.02	3.88	-----	0.23	0.12	0.07	0.06	0.06	0.12	0.46	0.29	0.08	0.17	0.22	0.18	0.09	0.28
16	0.10	0.18	0.53	0.56	0.39	8.72	0.12	0.62	0.19	0.78	0.52	0.85	-----	0.15	0.31	0.27	0.46	0.33	0.23	0.36	0.30	0.12	0.60	0.47	0.21	0.59
17	0.25	0.43	0.80	1.24	0.47	24.53	0.21	1.44	0.31	483.1	3.21	1.80	1.42	-----	0.19	0.13	0.27	0.07	0.00	0.28	0.05	0.00	0.38	0.26	0.05	0.38
18	0.77	0.76	0.71	2.31	0.47	0.66	0.46	19.60	0.47	1.20	3.56	3.20	0.56	1.04	-----	0.02	0.02	0.12	0.17	0.27	0.14	0.24	0.17	0.12	0.13	0.27
19	0.93	10.43	0.93	3.12	0.51	0.91	0.66	13.79	0.74	2.30	16.25	3.98	0.68	1.75	16.25	-----	0.02	0.06	0.10	0.22	0.07	0.17	0.13	0.08	0.07	0.22
20	0.87	0.89	0.92	1.93	0.46	0.62	0.61	5.19	1.06	0.83	10.75	3.88	0.29	0.67	12.13	16.25	-----	0.17	0.23	0.26	0.19	0.34	0.12	0.05	0.17	0.26
21	0.33	0.40	0.54	2.29	0.31	1.11	0.62	3.42	0.44	56.60	$	1.89	0.52	3.21	1.79	3.68	1.27	-----	0.03	0.40	0.00	0.18	0.34	0.26	0.05	0.41
22	0.30	0.43	0.72	1.13	0.41	2.72	0.48	1.36	0.35	$	7.48	0.29	0.82	146.0	1.23	2.32	0.85	7.48	-----	0.30	0.00	0.02	0.34	0.24	0.05	0.39
23	1.75	10.75	8.00	0.39	1.53	0.93	0.53	0.52	0.90	0.53	0.52	0.62	0.45	0.64	0.69	0.91	0.72	0.37	0.57	-----	0.38	0.32	0.09	0.06	0.26	0.02
24	0.33	0.40	0.59	1.81	0.33	1.38	0.58	2.50	0.41	$	0.88	3.05	0.58	5.04	1.56	3.19	1.09	$	132.7	0.41	-----	0.13	0.34	0.26	0.05	0.40
25	0.21	0.36	0.70	0.68	0.43	$	0.38	0.80	0.27	13.92	$	1.19	1.89	$	0.81	1.24	0.49	1.14	13.50	0.54	1.74	-----	0.43	0.32	0.08	0.43
26	60.25	2876	2.89	0.60	0.91	0.46	1.01	0.82	2.45	0.48	1.04	0.90	0.17	0.41	1.21	1.65	1.89	0.48	0.48	2.67	0.48	0.33	-----	0.00	0.26	0.01
27	2.47	9.32	3.54	0.75	0.99	0.67	0.66	1.11	1.20	0.73	3.78	1.11	0.28	0.70	1.78	3.05	4.39	0.73	0.81	4.01	0.73	0.54	$	-----	0.20	0.05
28	0.53	0.62	0.74	1.81	0.55	1.80	0.72	2.04	0.39	5.25	5.25	2.59	0.93	5.28	1.70	3.55	1.27	5.25	5.25	0.70	5.25	2.83	0.73	1.00	-----	0.30
29	12.76	$	5.74	0.41	1.21	0.49	0.98	0.51	2.31	0.37	0.56	0.64	0.17	0.40	1.68	0.90	0.73	0.36	0.39	14.74	0.37	0.34	35.50	5.13	0.58	-----

KEY: (Sites listed from west to east along the South African coast)
1 Saldanha; 2 Melkbosstrand; 3 Sea Point; 5 Muizenberg; 7 Gansbaai; 8 Pearly Beach; 9 Die Dam; 10 Cape Agulhas; 11 Struisbaai; 12 Arniston; 13 Witsand; 14 Stilbaai; 15 Mossel Bay; 16 Hamburg; 17 East London; 18 Cintsa; 19 Morgan's Bay; 20 Qolora Mouth; 21 Mazeppa; 22 The Haven; 23 Hole in the wall; 24 Coffee Bay; 25 Hluleka; 26 Umngazi; 27 Port St Johns; 28 Mkambati; 29 Port Edward