POPULATION GENETICS OF THE GREEN SEA URCHIN, *STRONGYLOCENTROTUS DROEBACHIENSIS.*

by

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"[A population] is not a defined genetic entity, but rather a part of a genetic mosaic fluctuating in time."

- David *et al.* 1997

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ABSTRACT

I used microsatellite genotypes and mitochondrial DNA sequences to study the population genetics of the green sea urchin (*Strongylocentrotus droebachiensis*) in the northwest Atlantic Ocean. I developed microsatellite markers for *S. droebachiensis* that are also suitable for genetic studies in *S. purpuratus*. I measured within and among population genetic differentiation at four microsatellite loci in eleven populations throughout the north Atlantic and northeast Pacific. I found small but significant genetic subdivision at the largest spatial scale ($F_{ST} = 0.0625$, $P = 0.0002$). One northwest Atlantic population recently affected by disease was significantly differentiated from some others, but otherwise there was little differentiation among populations within Atlantic Canada. All of these populations were highly inbred ($F_{IS} = 0.1499$).

I used COI haplotype data to investigate the phylogeographic history of populations in the northwest Atlantic, and to understand the relative influence of gene flow from Pacific and northeast Atlantic populations on the genetic variation in northwest Atlantic populations. Using analysis of molecular variance, statistical parsimony, mismatch, and nested clade analyses I found strong evidence of the survival of northwest Atlantic populations during recent Pleistocene glaciations, limited gene flow across the north Atlantic, and introgession of haplotypes from the congener *S. pallidus*.

I used an age class analysis of both microsatellite genotype and mitochondrial haplotype data to address the possibility that sea urchins are susceptible to a large variance in reproductive success. Significant genetic variation among age classes supported the hypothesis that reproduction in sea urchins can be viewed as a sweepstakes in which relatively few adults contribute to each spawning event.

I reviewed the effect of reproductive variation on the non-random component of inbreeding (F_{IS}). F_{IS} values were significantly associated with the mode of male spawning: by release of planktonic sperm versus some form of direct sperm transfer to females or benthic egg masses. Such free-spawning often produces low fertilization rates and highly variable fertilization success among individual males. This broad pattern among species is consistent with my observations of population genetic variation within *S. droebachiensis*, and suggests that the population genetic consequences of free-spawning could be widespread and significant.

ABBREVIATIONS AND SYMBOLS

°C	degrees Celsius
5'	the fifth carbon in the pentose group deoxyribose
3'	the third carbon in the pentose group deoxyribose
AMOVA	analysis of molecular variance
BLAST	Basic Local Alignment Search Tool
bp	base pair; 1 bp = 1 pair of nucleotides
COI	cytochrome *c* oxidase subunit I; a gene in the mtDNA genome
CTAB	cetyltrimethylammonium bromide
D_C	clade distance; the geographic range of a clade
ddH_2O	distilled de-ionized water
D_N	nested clade distance; the geographic range of the clade relative to other clades in the nested group
DNA	deoxyribonucleic acid
dNTP	Deoxyribonucleotide
F_{IS}	inbreeding coefficient; reduction in heterozygosity within individuals with respect to the subpopulation.
F_{IT}	reduction in heterozygosity within individuals relative to the total population.
F_{ST}	fixation index; reduction in heterozygosity within subpopulations relative to the total population.
FSTAT	*F*-statistic genetic analysis software
GDA	genetic data analysis software
GenBank	database of nucleotide sequences from more than 130,000 organisms
GENEPOP	population genetic analysis software
HCL	hydrochloric acid
H_E	expected heterozygosity
HKY85+G+I	The DNA substitution model of M. Hasegawa, H. Kishino, and T. Yano
H_O	observed heterozygosity
HWE	Hardy-Weinberg expectation of genotype frequencies
IRD	infrared dye

IRD700	infrared dye that fluoresces at a wavelength of 700nm
IRD800	infrared dye that fluoresces at a wavelength of 800nm
KCL	potassium chloride
km	kilometer; 1 km = 1000 meters
M	molar; unit of concentration; moles of solute per liter of solution
M13	filamentous bacteriophage of the bacterium *E. coli* used for cloning and sequencing
$MgCl_2$	magnesium chloride
µl	microliter; 1 µl = 10^{-6} liters
mm	millimeter; 1 mm = 10^{-3} meters
mM	millimolar; unit of concentration; 1mM = 10^{-3} M
mol	mole; the amount of substance that contains the same number of formula units as there are ^{12}C atoms in 12 grams of ^{12}C (6.0225×10^{23}, or Avogadro's number)
m s^{-1}	meters per second
mtDNA	mitochondrial DNA
Mya	million years ago
My	million years
NCA	nested clade analysis
Ne	effective population size
NE	northeast
ng	nanogram; 1 ng = 10^{-9} g
NW	northwest
pmol	picomole; 1 pmol = 10^{-12} moles
^{32}P	the radionuclide phosphorous-32
PCR	polymerase chain reaction
Φ_{CT}	F_{ST} analogue of among region variation calculated using a 3 level AMOVA
Φ_{RT}	F_{ST} analogue of within region variation calculated using a 3 level AMOVA

Φ_{ST}	F_{ST} analogue of among subpopulation variation calculated using a 2 level AMOVA
P	probability of significant departure from the null hypothesis
PAUP*	Phylogenetic Analysis Using Parsimony
pUC19/pUC18	engineered plasmid DNA used for cloning
R^2	correlation coefficient
RFLP	restriction fragment length polymorphism
s	second
SCUBA	Self Contained Underwater Breathing Apparatus
T_A	primer annealing temperature
Tau	the mode of the mismatch distribution
TBE	Tris Boric acid Ethylenediamine tetra-acetic acid
TCS	a computer program that estimates gene genealogies
Theta-0	parameter of the mismatch distribution; initial population size
Theta-1	parameter of the mismatch distribution; final population size
UPGMA	unweighted pair-group method of arithmetic averages

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I am grateful to the members of my supervisory committee: Mark Johnston, Robert Latta, and Robert Scehibling, for contributing to the growth of this research and for adding valuable biological context. I also thank Jeff Hutchings and Rob Toonen for comments on Chapter Six, and Meredith Trotter for an enlightening computer simulation.

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CHAPTER ONE

INTRODUCTION

Population genetic studies of marine organisms aim to identify the processes that generate and maintain genetic variation within a species, and to determine the temporal and spatial scales over which these processes operate. Marine population geneticists have focused on the biology of dispersal, variation in dispersal ability among species, and the influence of dispersal variation on population genetic structure. A major motivation for this focus on dispersal is the potential tradeoff between the levels of gene flow among populations and the potential for local differentiation and adaptation, which may be a precursor to speciation. One of the most well established patterns in marine population genetics is the relationship between duration of larval development in the plankton and the magnitude of genetic substructure: population genetic differentiation is higher when dispersal is inferred to be lower (e.g., Bohonak, 1999). The amount of gene flow among populations governs the rate at which random (i.e., genetic drift) and deterministic (i.e., natural selection) processes generate patterns of genetic differentiation within and among populations (Grosberg and Cunningham, 2001). However, since the size of populations and the magnitude of movements among them predict the rate at which equilibrium between drift and selection is achieved, it has been suggested that few (if any) marine species ever reach genetic equilibrium (see Grosberg and Cunningham, 2001). If patterns of population size and gene flow are not stable for sufficiently long periods to achieve this equilibrium, then population substructure might be more productively interpreted in

the light of biogeographic history (e.g., founder effects, range expansion) rather than in terms of contemporary patterns of gene flow (Bohonak, 1999).

I use microsatellite DNA and cytochrome *c* oxidase subunit I (COI) sequence data to address these population genetic issues in the green sea urchin (*Strongylocentrotus droebachiensis*). These sea urchins are free-spawners with long lived planktonic larvae that are capable of extensive dispersal (e.g. Strathmann, 1978). Several *Strongylocentrotus* species occur in the north Pacific, but only *S. droebachiensis* and *S. pallidus* have invaded the north Atlantic since the opening of the Bering Seaway about 3.5 Mya (Vermeij, 1991). In the northwest Atlantic, *S. droebachiensis* is frequently abundant and can play a critical role in the local community dynamics (e.g., Miller, 1985). A unique feature of the sea urchin populations along the coast of Nova Scotia is that they are periodically affected by disease epidemics. Shallow water populations can become infected with a parasitic amoeba (*Paramoeba invadens*) and occasionally mortality rates can be near 100% (e.g. Scheibling and Hennigar, 1997). Population surveys indicate that the return of sea urchins in at least some populations is mostly driven by recruitment of planktonic larvae (e.g. Schiebling and Raymond, 1990). Finally, some sea urchin populations have recently been targeted for commercial harvesting. This system provides the opportunity to examine both the spatial and temporal scale of genetic subdivision to address the population genetic consequences of the dispersal biology and other aspects of the early life history and biogeography of this sea urchin.

In order to characterize the levels of within and among population genetic differentiation I developed a unique set of genetic markers for the green sea urchin. Some other genetic studies of *Strongylocentrotus* sea urchins used allozyme electrophoresis (Edmands *et al.*, 1996; Moberg and Burton, 2000). These studies revealed low levels of

polymorphism across loci and a general lack of genetic substructure across an extensive portion of each species' range. Since low levels of genetic polymorphism can obscure genetic substructure (e.g., Wright and Bentzen, 1994), I chose to develop a set of hyper-variable microsatellite DNA markers. After an extensive DNA cloning and sequencing effort I was able to characterize four microsatellite loci to use in studies of *S. droebachiensis* and its congeners. I describe these markers in Chapter Two.

In Chapter Three I use these microsatellites to characterize the spatial genetic structure in eight northwest Atlantic populations, two populations from the northeast Atlantic, and one population from the Pacific. The results are consistent with the predictions of genetic homogeneity caused by planktonic larval dispersal, but the inbreeding coefficients were very high for the northwest Atlantic populations. This striking pattern suggests a departure from equilibrium population genetic conditions, and two intriguing aspects of this result shaped the remainder of my thesis.

First, Bohonak (1999) suggested that non-equilibrium population genetic patterns should be viewed in the context of the historical processes that shaped them. Patterns of pairwise genetic differentiation among populations sampled in the both the northeast and northwest Atlantic and the Pacific suggested that populations of sea urchins in the northwest Atlantic are more genetically similar to those in the Pacific than to the northeast Atlantic. This pattern was surprising because many marine invertebrate populations in the northwest Atlantic became locally extinct during the last glacial maximum and were recolonized from refugial populations in the northeast Atlantic (Wares and Cunningham, 2001). However, it is also known that sea urchins recently re-invaded the north Atlantic from the ancestral populations in the Pacific (Palumbi and Wilson, 1990; Palumbi and Kessing, 1991). In order to further address these observations

and provide some historical context to the population genetics of sea urchins in the northwest Atlantic I extended the microsatellite analysis to include COI sequence data. I present this analysis in Chapter Four. The temporal information contained in the gene genealogy provided a historical perspective and suggested that although there has been recent genetic contact across the north Atlantic, the sea urchins in the northwest Atlantic are largely descendents of a trans-Arctic re-invasion from the Pacific.

Second, the positive inbreeding coefficients observed in the microsatellite data indicate an excess of homozygous genotypes. I also observed many homozygote excesses in other free-spawning marine invertebrates during a literature survey I conducted while developing the microsatellites. In this case, fewer heterozygote genotypes are observed than are predicted by the allele frequencies. Although several explanations for this pattern have been suggested (e.g. Zouros and Foltz, 1984), it is possible that heterozygote deficits and the departure from equilibrium genotype frequencies could be generated by a Wahlund effect (Hartl and Clark, 1997). The classic Wahlund effect is produced when population samples include some undetected spatial genetic differentiation among groups that are included in the same sample. This is an unlikely explanation of heterozygote deficits in the green sea urchin: allele frequencies were homogeneous over a spatial scale of $\approx$ 2000 km. Dennis Hedgecock's (1994; Li and Hedgecock, 1998) observations of large variance in reproductive success suggest that a homozygote excess could result instead from a *temporal* Wahlund effect caused by the mixing of two or more genetically distinct cohorts within the same population sample. Under this hypothesis random oceanographic processes govern which subset of adult breeders successfully contribute to each breeding event. The predicted result is a small but significant genetic differentiation among cohorts of larvae as a result of random

genetic drift among breeding events. I address this possibility in Chapter Five by aging samples of sea urchins for which I had mtDNA haplotypes and microsatellite DNA genotypes. I observed a small but significant genetic differentiation among age classes, which suggests that the high inbreeding coefficients in multi-aged adult populations could be due to a temporal Wahlund effect.

In Chapter Six I extended this consideration of the temporal Wahlund effect in a literature review that focuses on inbreeding coefficients and reproductive biology in a diverse range of marine invertebrates. I was prompted to evaluate the literature in this context by the frequent observation of unexpectedly high inbreeding coefficients in free-spawning marine invertebrates. The review shows that, on average, species in which males free-spawn their sperm had higher inbreeding coefficients that those that had some form of copulation or direct sperm transfer. This result is striking because similar patterns were not evident when I compared inbreeding coefficients with respect to differences in female spawning ecology or the dispersal ability of larvae.

I end the thesis with a summary (Chapter Seven) of the major conclusions and insights from my research with respect to the general goals of marine population genetics research. These insights include the influence of several historical and non-equilibrium processes on marine invertebrate population genetics, the potential importance of variance in reproductive success as a mechanism producing non-equilibrium population genetic patterns, and the advantages of using multiple classes of genetic markers for studying such patterns. I use these insights to argue for a change in focus from equilibrium analyses of dispersal variation in marine organisms to an emphasis on historical effects, spawning ecology, and other non-equilibrium processes as the focus of marine population genetics research.

CHAPTER TWO

CHARACTERIZATION OF MICROSATELLITE LOCI IN SEA URCHINS (*STRONGYLOCENTROTUS* SPP.)

Molecular Ecology Notes (2002) 2, 493-493

Introduction

Sea urchins (*Strongylocentrotus* spp.) are ecologically and economically important throughout the shallow subtidal waters of the northwest Atlantic and northeast Pacific. Extreme fluctuations in the populations of *S. droebachiensis* in the northwest Atlantic may be caused by both frequent disease outbreaks (Scheibling and Hennigar, 1997) and commercial harvesting practices (Hatcher and Hatcher, 1997) followed by recruitment of widely dispersed planktonic larvae. Genetic structure in *S. droebachiensis* may be affected by disease epidemics, harvesting, and genetic drift associated with lottery-like reproductive variation. Microsatellite markers have been developed for similar investigations in the sea urchin *Evechinus chloroticus* (Perrin and Roy, 2000), and both population genetics and genome mapping in *S. purpuratus* (Cameron *et al.*, 1999). Here I describe a unique set of polymorphic microsatellite loci I use in population genetic studies of *S. droebachiensis*.

Methods and results

Genomic DNA was extracted from the gonad tissue of a single sea urchin (*S. droebachiensis*) using a CTAB (cetyltrimethylammonium bromide) buffer and phenol-

chloroform extraction (Grosberg *et al.,* 1996). The DNA was digested to completion using four restriction enzymes (*Alu*I, *Hae*III, *Hinc*II, and *Rsa*I) and size fractioned in an agarose gel. Fragments (300-700bp) were excised and purified using a standard phenol-freeze fracture protocol (Ausubel *et al.,* 1999) and ligated into the *Sma*1 site of pUC19 (Pharmacia). The library was transformed into *Escheria coli* (DH5α Gibco BRL Maximum Efficiency) and screened with ^{32}P-labeled $(GT)_{15}$ and $(GA)_{15}$ oligonucleotide probes. One hundred and eighty recombinant clones were isolated and sequenced in both directions using IRD labeled universal M13 primers and resolved in 8% (25cm, 0.25mm thick) denaturing polyacrylamide gels using a Li-Cor 4200 automated DNA sequencer.

All clones contained microsatellite repeats, however most were long (>40 repeats) and interrupted in several positions. BLAST searching did not suggest homology between these sequences and those previously reported for *S. purpuratus* (Cameron *et al.,* 1999). Primers were designed for 14 of the best sequences, one of which was 5' end labeled with either an IRD700 or IRD800 dye for visualization on the Li-Cor sequencer. Primer pairs were assayed on 10 individuals each of *S. droebachiensis* (collected from Halifax Harbor, Nova Scotia) and *S. purpuratus* (collected from Friday Harbor, Washington). Amplifications were performed in 5μl final volume containing 5-20 ng of template DNA, 10mM Tris-HCl, 50mM KCl, 0.01% gelatin, 0.1% Tween 20, 0.2 mM of each dNTP, 1.5mM $MgCl_2$, 1 pmol of each primer, and 0.25ul *Taq* Polymerase (MBI). The amplifications were performed using a Stratagene Robocycler with the following PCR profile: 94°C 60s, T_A°C 30s, 72°C 30s for 7 cycles and then 32 cycles of 90°C 30s, T_A°C 30s, 72°C 30s. The amplified products were resolved in 6% (25cm, 0.2mm thick)

denaturing polyacrylamide gels on the Li-Cor sequencer. Allele sizes were estimated using pUC18 DNA sequence fragments as standards.

A total of six primer pairs yielded interpretable amplification products in either *S. droebachiensis* or *S. purpuratus* (Table 2.1). The eight remaining loci all amplified in *S. droebachiensis*, however, at least one of the 10 individuals exhibited more than two alleles and these loci were deemed unsuitable for a population genetic analysis. Similar patterns were observed for two of the remaining eight loci in *S. purpuratus*, and six loci failed to amplify.

Four polymorphic loci were further characterized by scoring 100 individual *S. droebachiensis* collected from Norris Cove, Newfoundland (Table 2.1). Statistical analyses were performed using GENEPOP version 3.1d (Raymond and Rousset, 1995b). Three loci (*Sd*63, *Sd*76, *Sd*156) show significant deviations from Hardy-Weinberg equilibrium, suggesting the presence of null alleles. Segregating null alleles at *Sd*156 were observed in laboratory reared larvae (J. Ford, unpublished data), but the frequency of such nulls in the population are unknown. Since no homozygous nulls were detected in any of the 100 individuals sampled it is possible that the departures from Hardy-Weinberg equilibrium are a result of the Wahlund effect. All polymorphroic loci in *S. purpuratus* were in Hardy-Weinberg equilibrium.

Table 2.1 Characteristics of six microsatellite loci in the sea urchins *Strongylocentrotus droebachiensis* (N=100) and *S. purpuratus* (N=10). T_A, annealing temperature, P, optimal [primer] (pmol/5ul reaction), M, optimal [$MgCl_2$](mM), SR, size range in bp, A, number of alleles observed, H_E, expected heterozygosity, H_O, observed heterozygosity.

						S.droebachiensis				*S.purpuratus*			
Locus	Repeat	GenBank Accession No.	Primer Sequence (5'-3')	T_A (°C)	P, M	SR	A	H_E	H_O	SR	A	H_E	H_O
*Sd*52	$(GT)_{14}$	AF506800	A:CGGTATTGCAAAGCATAACAGG B:GAGTCACGTCTTTCGGATGG[2]	51	2, 3	MA[3]	-	-	-	129-155	10	0.92	0.70
*Sd*63	$(GA)_{12}$	AF506801	A:CTCTATGTGTCAATGTACCC B:TGTTGCGTAAATGGTGGGC[2]	49	1.5, 4	140-178	13	0.83	0.71[4]	NA[5]	-	-	-
*Sd*67	$(GT)_{12}$	AF506802	A:CAATCCCCAAACACCACCC[1] B:TTTCCCATTCTTCCTTCATCC	51	2, 2	117	1	-	-	109-117	5	0.44	0.30
*Sd*76	$(GT)_3GG(GT)_{14}$	AF506803	A:TAGTCATGTACATCAGTTGG B:ATTGTGATATGAAGGTGAGG[2]	49	1.5, 2	143-179	17	0.87	0.71[4]	141-159	6	0.81	0.70
*Sd*121	$(GA)_{26}$	AF506804	A:TTTAGGAATGGGTCAACTGG[2] B:CTATAGTTATCTTCTCAGTGG	49	0.5, 2	120-157	20	0.91	0.91	128-204	12	0.92	0.90
*Sd*156	$(GA)_{19}AG(GA)_2$	AF506805	A:TAAGTATACAGGTCGATTCG B:AGTAAAGTAAAATGCAGGCG[1]	49	1.5, 2.5	178-204	15	0.86	0.69[4]	174-198	9	0.89	0.80

[1]primer 5' labeled with IRD800 dye
[2] primer 5' labeled with IRD700 dye
[3]More than 2 alleles per individual were amplified
[4]Significant deviation from Hardy Weinberg expectation (P=<0.000, tested in GENEPOP 3.1a, Raymond and Rousset, 1995b)
[5]No products amplified

CHAPTER THREE

ANALYSIS OF POPULATION GENETIC STRUCTURE OF THE GREEN SEA URCHIN (*STRONGYLOCENTROTUS DROEBACHIENSIS*) USING MICROSATELLITES.

Marine Biology (2004) 144, 243-251.

Introduction

Green sea urchins, *Strongylocentrotus droebachiensis,* are ecologically and economically important members of the shallow water marine ecosystem of northern oceans. In many parts of this wide geographical range, the population biology of these animals is characterized by large fluctuations in adult abundance that are associated with intensive grazing of kelps, disease outbreaks, episodic recruitment of planktonic larvae, and commercial harvesting practices (Chapman, 1981; Pringle *et al.,* 1982; Miller, 1985; Johnson and Mann, 1988; Scheibling and Raymond, 1990; Hatcher and Hatcher, 1997). Green sea urchins have a high dispersal life history syndrome characterized by high fecundity, free spawning with external fertilization, and a planktonic larval stage that lasts from four to 21 weeks (Strathmann, 1978; Hart and Scheibling, 1988). The frequency and intensity of larval recruitment is strongly influence by this lengthy planktonic period. For example, such larvae spawned off the coast of Nova Scotia may be capable of travelling >1000 km before settlement, even in relatively slow surface currents such as the 0.05-0.10 m s^{-1} Nova Scotian current (Petrie, 1987). The majority of larvae in the northwest Atlantic settle in the summer months, but settlement is known to be highly variable in both space and time. Balch and Scheibling (2000) report orders of magnitude

differences in interannual settlement rates for *S. droebachiensis* in shallow waters off the Atlantic coast of Nova Scotia, and similar patterns have been observed in the Gulf of Maine (Harris *et al.,* 1994) and the Bay of Fundy (Balch *et al.,* 1998). While settlement pulses have been shown to correlate with high sea surface temperatures (Hart and Scheibling, 1988), Balch and Scheibling (2000) suggest that reproductive output and larval survival may be more important than environmental factors (e.g., temperature) in the generation of annual variation in settlement rates.

Species with the high dispersal life history syndrome are expected to generally fit a model of panmixia with little genetic divergence among populations compared to species with limited dispersal ability (see reviews by Burton, 1983; Bohonak, 1999). However, allozyme studies of marine invertebrates have demonstrated small but statistically significant levels of population subdivision without isolation by distance (e.g., limpets: Johnson and Black, 1982, 1984a, 1984b; crown-of-thorns starfish: Benzie and Stoddart, 1992; sea urchins: Marcus, 1977, Watts *et al.,* 1990; zebra mussels: Lewis *et al.,* 2000). In these cases the genetic differences between neighbouring populations often exceed those between more distant populations. Population genetic studies of both *S. purpuratus* using allozyme and mitochondrial DNA (mtDNA) (Edmands *et al.,* 1996; Flowers *et al.,* 2002) and *S. franciscanus* using allozymes (Edmands *et al.,* 1996) and nuclear DNA sequence data (Debenham *et al.,* 2000) have revealed similar patterns. The larger data sets analyzed in these studies improved the resolution of genetic differentiation and revealed patterns that were undetectable in previous analyses (e.g., Palumbi and Wilson, 1990; Palumbi, 1995).

Although the prolonged larval stage of *S. droebachiensis* is expected to facilitate high levels of gene flow, it is possible that other physical and biological features of the

northwest Atlantic ecosystem may promote population subdivision. For example, commercial harvesting (Hatcher and Hatcher, 1997) and disease outbreaks caused by the protozooan *Paramoeba invadens* (Scheibling and Hennigar, 1997) can both cause severe local population reduction in shallow waters. These localized population bottlenecks are followed by recruitment of planktonic larvae, and such episodic changes in population size may have important population genetic consequences. One initial goal of this study was to characterize the population genetic effects (if any) of spatially and temporally patchy disease outbreaks followed by variable patterns of larval recruitment. Spatially and temporally variable recruitment patterns are one potential source of unexpected population genetic differentiation in species with lengthy planktonic larval stages (Johnson and Black, 1984a).

Here I present four-locus microsatellite allele frequency data for eleven *S. droebachiensis* populations spanning >6000 km of the species range. I focus on populations from the northwest Atlantic because knowledge of the population biology and ecological interactions of sea urchins is particularly well studied in this region. I compare differentiation among populations in Atlantic Canada to differentiation across the north Atlantic and between the north Atlantic and north Pacific. Unlike other studies of *Strongylocentrotus* population genetics, I found evidence of strong population differentiation only at the largest spatial scales. This evidence is consistent with biogeographic hypotheses of Pleistocene range expansion into the north Atlantic from the north Pacific.

Materials and methods

Field sampling

Tube foot or gonad tissue was obtained from *Strongylocentrotus droebachiensis* collected between 1999 and 2002 from ten sites throughout the north Atlantic (Figure 3.1) and one site from the northeast Pacific. Sample sites were chosen to cover a wide portion of the species range in Atlantic Canada; specific sample locations were chosen based on accessibility and local knowledge of sea urchin occurrence. Two populations (at Bear Cove, west of Halifax) and East Jeddore (east of Halifax) were chosen because they experienced complete and near complete mortality, respectively, in 1995 as a result of disease outbreaks (Scheibling and Hennigar, 1997). These two sites are less than 50 km apart (straight line distance).

All samples were collected from depths ranging from three to fifteen meters using SCUBA. Sea urchins from San Juan Channel, Washington, were collected from a depth of about 50 meters by dredge. Tissue samples were stored in 95% ethanol. The test diameters of individual sea urchins ranged from a minimum of 20 mm to a maximum 100 mm. I sampled a range of test diameters in order to sample sea urchins of different ages (and recruitment events). However, variation in test growth rate and asymptotic growth in older individuals lead to a low correlation between test diameter and age within and among some populations (Meidel and Scheibling, 1998).

Molecular analysis

Genomic DNA from some sea urchins was extracted from tube feet by incubating a single sucker in 20 µl of distilled deionized water (ddH_2O) with 10 µg proteinase K (Qiagen) for 1 hr at 65°C. Samples were then incubated for 5 min at 80°C and 1 µl of this

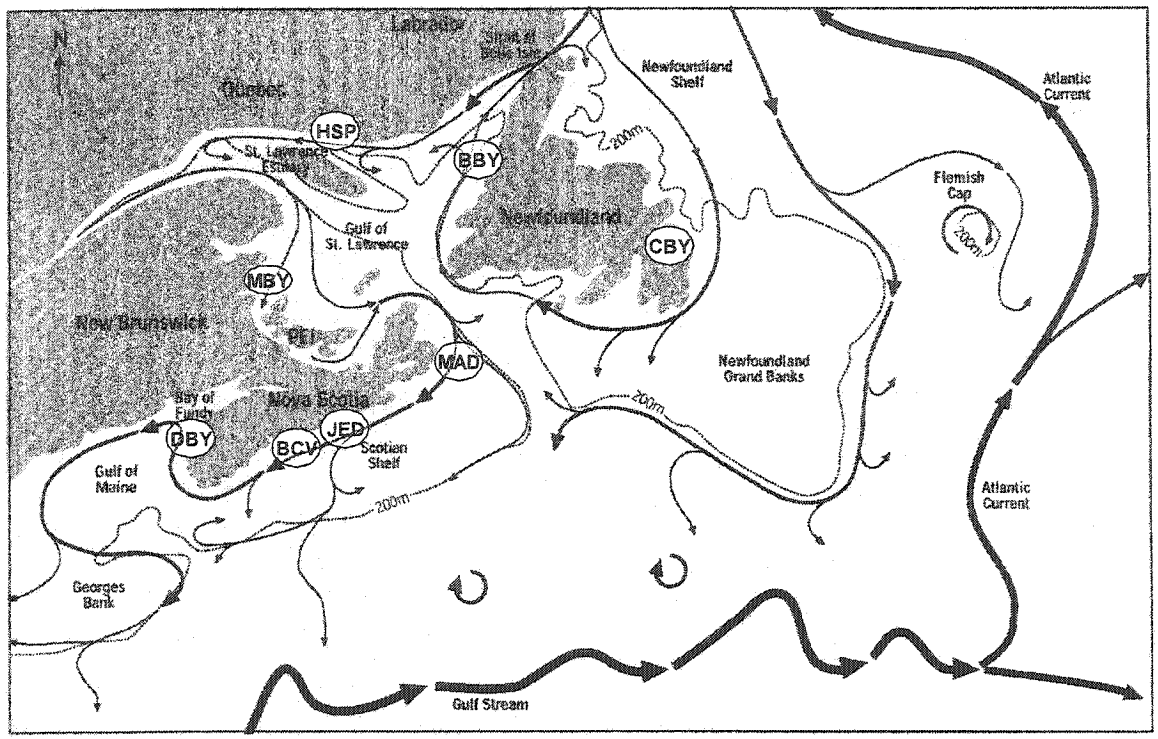

Figure 3.1 Sampling locations of shallow water populations in the northwest Atlantic Ocean. Arrows indicate average annual current directions and line thickness indicates average flow velocity (<0.005, 0.05-0.10, 2.5 m s^{-1}). Populations are: Conception Bay (CBY), Bonne Bay (BBY), Harve St. Pierre (HSP), Miramichi Bay (MBY), Main-a-dieu (MAD), East Jeddore (JED), Bear Cove (BCV), and Digby (DBY). See Table 1 for exact population locations. (Adapted with permission from the Nova Scotia Museum (http://museum.gov.ns.ca/mnh/nature/nhns/index.htm).

digest was used as template for each polymerase chain reaction (PCR). For most sea urchins, genomic DNA was isolated from gonad tissue using a cetyltrimethylammonium bromide (CTAB) buffer and phenol-chloroform extraction (Grosberg *et al.*, 1996). The two different extraction methods produced identical microsatellite genotypes. A total of 966 sea urchins (mean = 88 per population; range = 37 - 110) were characterized with the four microsatellite markers described in Chapter 2 (Addison and Hart, 2002). The PCR products were amplified using the Stratagene Robocycler and MJ PTC-100 thermal cyclers. Fragment sizes were resolved on a Li-Cor DNA 4200 following the methodology outlined in Chapter Two (Addison and Hart, 2002).

Data analysis

Allele frequencies, observed (H_O) and expected heterozygosity (H_E), and exact tests for the conformance to Hardy-Weinberg expectations (HWE) were calculated using the web version of GENEPOP (Version 3.1; Raymond and Rousset, 1995b). Linkage disequilibrium was calculated using Fisher's exact test by permuting all two-locus genotypes within all populations using Genetic Data Analysis (GDA vers 1.1; Lewis and Zaykin, 1996). Allelic richness was calculated for each population using FSTAT (Goudet, 2001) for the three loci in which no missing values were found.

To assess the levels of genetic differentiation within and among populations *F*-statistics (F_{IS}, F_{IT}, F_{ST}) were calculated for the eight northwest Atlantic populations and again for all eleven populations throughout the species range using GDA. Ninety-five percent confidence intervals were calculated for the *F*-statistics by bootstrapping across loci (number of replications = 10,000) using GDA. Pairwise F_{ST} were computed for all northwest Atlantic populations. A dendogram based on coancestry distances computed

by GDA was drawn using PAUP 4.0b10 (Swofford, 2002). Pairwise tests of multi-locus genotypic differentiation were conducted for all northwest Atlantic populations using randomization procedures and G tests for differentiation using FSTAT.

Results

Genetic variation within populations

All northwest Atlantic populations and the one Pacific population had high levels of genetic variability with many alleles at each locus and high observed heterozygosities (Table 3.1). The two northeast Atlantic populations (Iceland and Norway) had fewer alleles at each locus, and the locus *Sd*76 failed to amplify in all individuals from these two populations. Allelic richness in Iceland and Norway varied across loci from 3.69 to 7.19 (allelic richness was not calculated for *Sd76* because no alleles were amplified from northeast Atlantic samples). Allelic richness in all other population samples was 10.04-18.68. Significant departures from Hardy-Weinberg expectations were detected in 22 of the 42 possible tests (Table 3.1), and 18 of these were statistically significant after a table-wide Bonferroni correction for multiple tests (Rice, 1989). Positive F_{IS} values indicate the departure from Hardy-Weinberg expectations was a result of an excess in homozygosity. Jackknifing over all populations indicates that departures from Hardy-Weinberg expectations ($F_{IS} \pm$ S.D.) were significant for *Sd*156 (0.1878 ± 0.0237), *Sd*76 (0.2302 ± 0.0226), and *Sd*63 (0.1654 ± 0.0322), but not for *Sd*121 (0.0095 ± 0.0144). I have detected null alleles segregating in laboratory families at *Sd*156, but not at other loci (J. Ford, unpublished data). However, the widespread

Table 3.1 Sampling location (abbreviation), latitude and longitude, sample size (N), number of alleles (N_A), allelic size range (SR) in base pairs, and the expected (H_E)and observed (H_O) heterozygosity. Bold type indicates samples that deviate significantly from Hardy-Weinberg expectations after sequential Bonferroni corrections.

	Conception Bay, (CBY) 47°38'N, 52°50'W	Bonne Bay, (BBY) 49°31'N, 57°53'W	Havre St. Pierre, (HSP) 50°14'N, 63°36'W	Miramichi Bay (MBY) 47°08'N, 64°58'W	Main-a-dieu, (MAD) 46°00'N, 59°51'W	East Jeddore, (JED) 44°44'N, 63°00' W	Bear Cove, (BCV) 44°32'N, 63°33' W	Digby, (DBY) 44°37'N, 65°46'W	Vestfjorden Norway (67°21'N, 14°30'W)	Hvalfjordur Iceland (64°21'N, 21°29'W)	San Juan Channel, Washington (48°33'N, 123°01'W)
*Sd*156											
N	97	100	97	95	95	104	110	99	79	49	37
N_A	15	14	12	13	13	13	13	12	6	4	13
SR (bp)	172-204	178-204	178-202	178-202	178-204	178-202	178-202	178-202	167-194	182-196	178-206
H_E	0.91	0.86	0.88	0.88	0.88	0.86	0.89	0.88	0.23	0.28	0.89
H_O	**0.67***	**0.69***	0.77	**0.67***	0.79	**0.72***	0.77	**0.70***	0.23	0.24	0.89
*Sd*121											
N	97	100	97	95	95	104	110	99	79	49	39
N_A	20	20	20	22	18	22	19	21	7	7	17
SR (bp)	120-158	120-160	120-158	120-159	120-158	120-164	120-159	114-157	120-142	120-142	118-154
H_E	0.89	0.91	0.90	0.91	0.89	0.82	0.93	0.90	0.74	0.66	0.92
H_O	0.90	0.91	0.90	0.92	0.89	0.82	0.95	0.84	0.73	0.57	0.85

Table 3.1
continued

*Sd*76											
N	97	100	97	95	95	104	110	98	-	-	32
N_A	13	17	12	14	14	15	13	14	-	-	12
SR (bp)	145-171	145-179	145-171	145-171	145-179	145-179	145-179	145-171	-	-	145-171
H_E	0.87	0.87	0.87	0.88	0.87	0.85	0.87	0.87	-	-	0.88
H_O	**0.68***	**0.71***	**0.69***	**0.69***	**0.66***	0.73	**0.56***	**0.68***	-	-	0.81
*Sd*63											
N	97	100	97	95	95	104	110	99	79	49	41
N_A	12	13	12	15	12	13	14	14	6	8	12
SR (bp)	138-178	140-178	138-178	132-178	140-178	140-178	140-178	140-178	140-154	138-154	140-170
H_E	0.83	0.83	0.81	0.85	0.80	0.86	0.82	0.83	0.59	0.65	0.77
H_O	**0.62***	**0.71***	0.70	**0.63***	0.66	**0.58***	0.75	**0.69***	0.66	0.69	**0.51***

departures from Hardy-Weinberg expectations are not thought to be caused by null alleles because null/null homozygotes were conspicuously rare in this data set. Homozygote and heterozygote genotypes were easy to distinguish on gel images. Only one individual (from Digby) failed to amplify at one locus (*Sd*76), but PCR products were obtained for all remaining individuals in the northwest Atlantic. Failure rates were higher for all loci in the Pacific samples, but this could be due to the poor quality and limited quantity of these tissue samples. Null alleles were probably not common in this Pacific population because we detected significant heterozygote deficits at only one of four loci (compared to two or three significant deficits for most northwest Atlantic populations in which null homozygotes were completely absent). No linkage disequilibrium was detected between any loci in any of the populations (data not shown).

Genetic variation among populations

I found no statistically significant genetic variation among populations in the northwest Atlantic ($F_{ST} = 0.0016$, $P > 0.05$; Table 3.2). When I included all eleven populations sampled throughout the Atlantic and Pacific Oceans, the mean F_{ST} increased to 0.0870 ($P < 0.05$; Table 3.2). This value was significantly different from zero and its magnitude suggests a moderate amount of genetic subdivision at this large (>6000 km) spatial scale. When I removed *Sd*76 from the analysis the resulting mean F_{ST} based on the three-locus genotypes was reduced to 0.0625 ($P < 0.05$; Table 3.2).

In addition to these quantitative population differences, the failure of *Sd*76 to amplify in the northeast Atlantic populations suggests that there has been little recent gene flow across the north Atlantic Ocean: all northeast Atlantic individuals were null

Table 3.2 Wright's *F*–statistics calculated using the methods of Weir and Cockerham (1984) in GDA. Comparisons are for individual loci in the northwest Atlantic and both three- and four-locus analysis for all eleven populations sampled throughout the range. Ninety five percent confidence intervals were calculated by bootstrapping across loci (no. reps = 10,000) (*$P < 0.05$).

Analysis	Locus	F_{IS}	F_{IT}	F_{ST}
NW Atlantic	*Sd*156	0.1959	0.1977	0.0023
	*Sd*121	-0.0020	-0.0031	-0.0011
	*Sd*76	0.2269	0.2263	-0.0008
	*Sd*63	0.1855	0.1907	0.0063
NW Atlantic	Mean	0.1499*	0.1514*	0.0016
All populations 4 loci	Mean	0.1436*	0.2181*	0.0870*
All populations *Sd*76 removed	Mean	0.1169*	0.1721*	0.0625*

homozygotes at this locus, and I found only one null homozygote at this locus in all other populations. Any significant flux of larvae from west to east should introduce amplifiable *Sd76* alleles into Iceland and Norway. Migration from east to west (Wares and Cunningham, 2001) could introduce null alleles into northwest Atlantic populations, and it is possible that such null alleles contribute to the deficit of *Sd76* heterozygotes in Atlantic Canada (see below). However, such migration does not appear to explain heterozygote deficits at other loci in the same populations.

The UPGMA tree based on pairwise F_{ST} values among all eleven populations clearly indicates the genetic subdivision between the northwest and northeast Atlantic populations of sea urchins, and the close relationship between the Pacific sample and those from the northwest Atlantic (Figure 3.2). Pairwise tests of genotypic differentiation revealed three cases in which two populations had significantly different genotype frequencies after a Bonferroni correction (Table 3.3). These three cases all involved the population sampled from East Jeddore which experienced near complete mortality as a result of a disease epidemic in 1995 (Scheibling and Hennigar, 1997), and pairwise differences were marginally non-significant in three of the other four comparisons involving this population. No similar patterns were observed for samples collected at Bear Cove, which experienced complete mortality in 1995. These differences therefore provide no consistent evidence of an effect of disease-induced population fluctuations on population genetic differentiation.

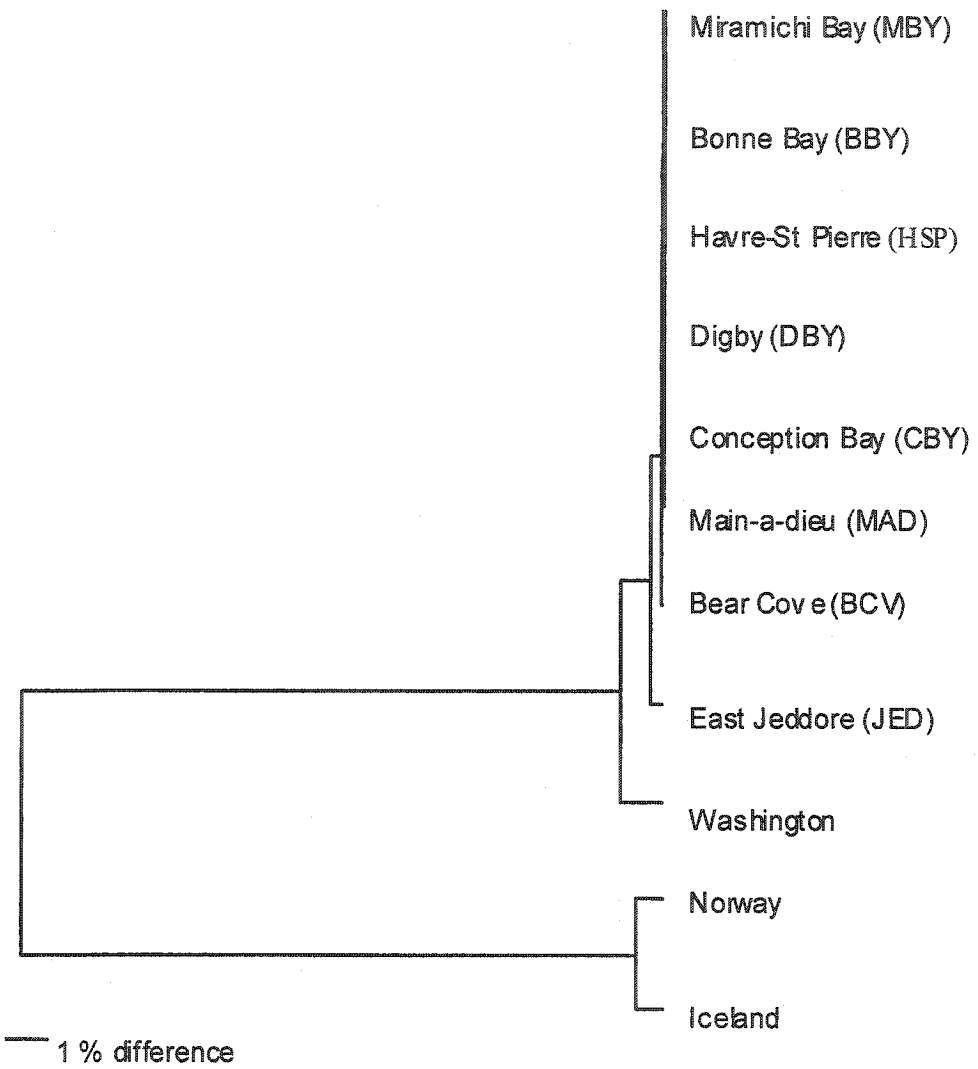

Figure 3.2 UPGMA dendrogram of all 11 populations of *Strongylocentrotus droebachiensis* based on four-locus microsatellite coancestry distances (pairwise F_{ST}) calculated using GDA. A dendrogram based on Nei's D has the same topology and similar branch lengths.

Table 3.3 Population pairwise F_{ST} values calculated for northwest Atlantic populations using GDA (upper diagonal) and *P*–values for calculations of significant pairwise genotype differentiation calculated using FSTAT (lower diagonal). *P*-values in bold represent significant differences after Bonferroni corrections for multiple tests. Populations abbreviations are as follows: Conception Bay (CBY), Bonne Bay (BBY), Havre St. Pierre (HSP), Miramichi Bay (MBY), Main-a-dieu (MAD), East Jeddore (JED), Bear Cove (BCV), and Digby (DBY).

Sample Site	CBY	BBY	HSP	MBY	MAD	JED	BCV	DBY
CBY		0.0012	-0.0006	-0.0013	0.0008	0.0105	-0.0002	0.0000
BBY	0.3893		0.0007	0.0001	-0.0002	0.0052	0.0017	-0.0009
HSP	0.2000	0.2857		0.0000	-0.0014	0.0055	-0.0008	-0.0009
MBY	0.3821	0.5786	0.2339		0.0012	0.0043	0.0015	-0.0014
MAD	0.1982	0.7464	0.7357	0.0714		0.0041	0.0002	-0.0004
JED	**0.0012**	0.0375	**0.0018**	0.1214	0.0536		0.0086	0.0040
BCV	0.1554	0.5964	0.0125	0.0679	0.1643	**0.0018**		0.0011
DBY	0.0339	0.7054	0.2214	0.4750	0.6196	0.0107	0.0429	

Discussion

Variation within the northwest Atlantic

The populations of green sea urchins (*Strongylocentrotus droebachiensis*) sampled throughout the northwest Atlantic Ocean are genetically homogeneous (F_{ST} is not significantly different from zero). This is consistent with a high level of gene flow or a low rate of genetic drift, and suggests that populations on this spatial scale act as one large interbreeding unit. Samples collected from disease affected populations (East Jeddore and Bear Cove) only 50 km apart had the highest pairwise genetic differences among all the comparisons made. These patterns are similar to the "chaotic genetic patchiness" observed for a variety of benthic marine invertebrates with the high dispersal life history syndrome (e.g., Johnson and Black, 1982; see review by Hellberg *et al.*, 2002). In these cases populations were genetically homogeneous over a large spatial scale, but included some statistically significant small-scale genetic heterogeneity. In this study, one population with a recent history of disease outbreak and population decline (East Jeddore) was genotypically distinct in pairwise comparisons, but others (e.g., Bear Cove) were not (Scheibling and Hennigar, 1997). Some strong differences among populations recently affected by disease may reflect sporadic and unpredictable recruitment (Harris *et al.*, 1994; Balch *et al.*, 1998) and lack of older individuals at these sites. However, these data do not strongly support the hypothesized effect of population fluctuation on local population genetic differentiation. The effect of population decline and subsequent larval recruitment on genetic differentiation requires more detailed studies of the genetic composition of new recruits over several recruitment events (Chapter Five).

Although mtDNA and sperm bindin sequence polymorphisms suggest genetic homogeneity of *Strongylocentrotus* populations on the west coast of North America

(Palumbi and Wilson, 1990; Debenham *et al.*, 2000; Flowers *et al.*, 2002), the lack of genetic substructure in this study is surprising because the allozyme studies of sea urchins sampled at similar spatial scales reveal significant levels of genetic heterogeneity (Marcus, 1977; Watts *et al.*, 1990; Edmands *et al.*, 1996; Mladenov *et al.*, 1997; Moberg and Burton, 2000) using much less polymorphic genetic markers. Moberg and Burton (2000) reported $F_{ST} = 0.033$ for *S. franciscanus* adults from the eastern Pacific coast of North America. Remarkably, Edmands *et al.* (1996) reported the same value ($F_{ST} = 0.033$) for *S. purpuratus* adults sampled from the same coastline. Both estimates were significantly greater than zero. The nominal level of subdivision in these two species is much larger than that found in this study ($F_{ST} = 0.0016$; Table 3.2). Because all three species share similar larval biology and ecology, I expected to detect similar levels of genetic substructure. Burton (1983) suggested that where the potential for extensive larval dispersal exists then populations should have no capacity to differentiate on the scale of typical larval dispersal, and thus significant genetic subdivision at or below this geographic scale must be driven by natural selection. Differences in oceanographic conditions and patterns of surface currents on each coast may be responsible for the differences in population structure, but the potential influence of natural selection in the allozyme studies or of non-equilibrium population processes in this study cannot be ruled out.

Population genetic studies of cod (*Gadus morhua*; Bentzen *et al.*, 1996; Pogson *et al.*, 2001; Beacham *et al.*, 2002; Knutsen *et al.*, 2003) and herring (*Clupea harengus*; McPherson *et al.*, 2001) in the northwest Atlantic using microsatellite markers have also demonstrated small but significant levels of genetic subdivision. However, microsatellite analysis of scallops (Herbinger *et al.*, 1998) and barnacles (Dufresne *et al.*, 2002)

throughout this same region fail to reveal any significant genetic heterogeneity. The consistent lack of genetic structure among these studies and my own suggest that the passive larval dispersal and sedentary adult lifestyle of many marine invertebrates may limit the formation of population substructure. In contrast, behavioral associations in fish, such as schooling or spawning site fidelity, may be responsible for the formation of the genetically distinct assemblages that are frequently detected.

Heterozygote deficits

Populations sampled in the northwest Atlantic had high inbreeding coefficients (mean $F_{IS} = 0.1499$, $P < 0.05$) and consistent heterozygote deficits were detected for three of the four loci used (*Sd*156, *Sd*76, and *Sd*63; Tables 3.1, 3.2). It is unlikely that null alleles are the cause of these deviations because only one possible null homozygote was detected among 797 individuals screened. Inbreeding is also an unlikely explanation because high dispersal is predicted to reduce the probably of mating among close relatives (reviewed by Bohonak, 1999). While a correlation between allele frequencies at neutral loci and some allozyme alleles under selection has been demonstrated in the acorn barnacle (*Semibalanus balanoides*) (Dufresne *et al.,* 2002), possible selective forces that could act on linked loci in the green sea urchin across the wide geographic range sampled in this study are unknown. Selection could generate heterozygote deficiencies (e.g. Zouros and Foltz, 1984), but selection is an unlikely explanation of the high inbreeding coefficients detected at multiple loci in green sea urchins.

An alternate explanation for these high inbreeding coefficients is based on the hypothesis that populations of marine free spawners experience a large variance in reproductive success (Hedgecock, 1994). The chance matching of reproductive effort to

oceanographic conditions that promote fertilization, growth, development, and settlement of larvae is likely a random process in which the likelihood of reproductive success is independent of genotype. The two main predictions of this model are that cohorts should be genetically differentiated from each other, and each cohort should have reduced allelic diversity because few adults in the population contributed successfully to the resulting pool of offspring (Hedgecock, 1994; Li and Hedgecock, 1998). If cohorts are temporally and spatially differentiated, then an age structured population should demonstrate unrecognized temporal structure as a kind of Wahlund effect. Since the spawning populations sampled in this study are genetically homogeneous, the large deficits in heterozygotes are unlikely due to a spatial Wahlund effect. Instead I suggest that my results are consistent with the prediction that large deficits in heterozygotes result from a temporal Wahlund effect caused by a large variance in reproductive success. Supporting evidence for this argument is the observation of small scale genetic differentiation between geographically close populations in an otherwise panmictic population. It is unlikely that temporal variation in the sources of new recruits could alone account for small scale genetic differentiation between East Jeddore and Bear Cove. The observation of equilibrium genotype frequencies in northeast Atlantic populations is not consistent with the hypothesis of a reproductive variance, and suggests either that these populations experience reproductive conditions that promote low variance in reproductive success or that the temporal Wahlund effect is not a general explanation of within-population genetic variation in these and other sea urchins (e.g., Flowers *et al.*, 2002).

Population structure across the species range

The significant F_{ST} of 0.0870 ($P < 0.05$) that was calculated among all eleven populations suggests that geographic subdivision in this species is manifest only on a very large spatial scale. The failure of *Sd*76 to amplify in the northeast Atlantic populations further suggests that these populations are not currently connected by gene flow to populations in the northwest Atlantic. The failure of this locus to amplify in some populations also highlights the potential pitfalls of employing hyper-variable markers such as microsatellites across a broad geographic range in which there are potentially deep divergences between populations that are conspecific but genetically isolated from each other on large spatial and temporal scales.

Fossil records indicate that *S. droebachiensis* invaded the Atlantic from the Pacific after the Bering Seaway opened 3.5 Mya (Durham and MacNeil, 1967). It has been suggested that, as a result of more recent climate change, north Atlantic populations may have undergone recent extinctions or severe bottlenecks followed by reinvasion from the Pacific. Both Palumbi and Wilson (1990) and Palumbi and Kessing (1991) suggest recent, but not continuous, migration of *S. droebachiensis* from the Pacific to the northwest Atlantic in the last 0.3 – 0.09 My. Extensive outflow of freshwater along the Arctic coast of Siberia is thought to have restricted the trans-Arctic interchange to the Canadian Arctic and not the Eurasian Arctic (Gladenkov, 1979). There is evidence that the magnitude of the extinctions caused by Pleistocene glacial advances were less severe in the northeast Atlantic than in the northwest Atlantic because of the presence of rocky substratum offering glacial refugia to the south (Vermeij, 1991). My results are consistent with these hypotheses of historical phylogeography. The lack of amplification at *Sd*76, and the reduced number of alleles in the northeast Atlantic, suggests that these

populations experienced a severe bottleneck (and became fixed for one or more null alleles at *Sd*76) but were not recently recolonized from the Pacific. In spite of the low genetic diversity within both of these populations at all loci (4-8 alleles, compared to 12-22 alleles in all other populations), I found no heterozygote deficits in the northeast Atlantic. Both of these patterns are consistent with a historical population reduction followed by restoration of equilibrium genotype frequencies in the absence of detectable gene flow from the Pacific or the northwest Atlantic populations. In contrast, the relatively high polymorphism in the northwest Atlantic and genetic similarity to the Pacific population indicate either glacial refugia or extinction followed by more recent and extensive recolonization from the Pacific. The failure to find genetic evidence of glacial refugia in several other obligate rocky substratum inhabitants in the northwest Atlantic supports the local extinction argument (Wares and Cunningham, 2001). However, contrary to Wares and Cunningham (2001), my results do not suggest recent recolonization from northeast Atlantic populations. Evidence of such a recolonization event would include lower diversity of alleles in the northwest Atlantic compared to the northeast Atlantic, a pattern opposite to my observations.

CHAPTER FOUR

COLONIZATION, DISPERSAL, AND HYBRIDIZATION INFLUENCE PHYLOGEOGRAPHY OF NORTH ATLANTIC SEA URCHINS (*STRONGYLOCENTROTUS DROEBACHIENSIS*).

Introduction

Quaternary sea level fluctuations and climate change have influenced the genetic structure and evolutionary history of many north Atlantic marine organisms (e.g., van Oppen *et al.*, 1995; Dahlgren *et al.*, 2000; Wares and Cunningham, 2001; Väinölä, 2003). Many species first arrived in the north Atlantic from the Pacific soon after the Bering Seaway opened 3.5 Mya (Durham and MacNeil, 1967; Vermeij, 1991). Following these invasions, eustatic sea level changes during the Pleistocene ice ages (2.4 My) are thought to have periodically closed these migratory routes causing widespread isolation and vicariance in the north Atlantic (*see* Cunningham and Collins, 1998; Hewitt, 1996). These glacial cycles are approximately 0.1 My in length and have dominated the climate of the northern hemisphere over the last 0.7 My (Hewitt, 1996). The last glacial maximum (c.a. 0.02 Mya) was thought to have severely affected the population biology of obligate rocky intertidal species of the northwest (NW) Atlantic because glaciers likely covered the full extent of their rocky habitat (Wares and Cunningham, 2001). For example, a comparative phylogeographic analysis revealed that several species in the NW Atlantic were extirpated and subsequently recolonized from glacial refuges in the northeast (NE) Atlantic (Wares and Cunningham, 2001). Estimated times of colonization calculated using coalescent methods were concordant with a range expansion after the last

glacial maximum. However, the much longer coalescent times for a barnacle (*Semibalanus balanoides*), a mussel (*Mytilus edulis*), and a sea star (Wares, 2001) were consistent with patterns of glacial refugia in the NW Atlantic (rather than extirpation and recent recolonization). Wares and Cunningham (2001) suggest that NW Atlantic populations of these species persisted because their long lived planktonic larvae were capable of quickly dispersing into suitable habitat during periods of rapid environmental change such as fluctuations in glacial extent and the appearance or elimination of suitable rocky habitats.

My study of microsatellite variation in green sea urchins (*Strongylocentrotus droebachiensis*) suggests that populations in the NW Atlantic are more closely related to those in the Pacific than to populations in the NE Atlantic (Chapter Three; Addison and Hart, 2004). The biogeographic history of NW Atlantic populations of sea urchins is further complicated by a relatively recent trans-Arctic connection to ancestral Pacific populations. Although there is evidence of trans-Atlantic gene flow inferred from sea urchin microsatellites, the pattern of population genetics in the green sea urchin is consistent with either glacial refugia in the NW Atlantic or extinction followed by extensive recolonization from the Pacific (rather than recolonization from refuges in the NE Atlantic). Recent gene flow from the Pacific has been detected for several marine species (Meehan *et al.*, 1989; Taylor and Dodson, 1994; Rawson and Hilbish, 1995; van Oppen *et al.*, 1995; Väinölä, 2003) including *Strongylocentrotus droebachiensis* (Palumbi and Wilson, 1990) and *S. pallidus* (Palumbi and Kessing 1991). These studies indicate that climate warming during interglacial periods promoted both trans-Arctic and trans-Atlantic dispersal of sea urchins, but the relative influence of these events on population genetic structure in the NW Atlantic are unknown.

The use of mitochondrial DNA (mtDNA) sequences has in many cases revealed genetic breaks that correspond to historic barriers to dispersal caused by lower sea levels during glacial periods (see Avise, 2000). Nested clade analysis (NCA) can be used to interpret the geographical associations of genealogical lineages and to disentangle population structure from population history (Templeton *et al.*, 1995; Templeton, 1998). This methodology has successfully resolved patterns of gene flow and historic genetic subdivision in other marine invertebrates including the sponges *Lucetta chagosensis* (Wörheide *et al.*, 2002) and *Crambe crambe* (Duran *et al.*, 2004), and the sea cucumber *Holothuria nobilis* (Uthicke and Benzie, 2003).

I used mtDNA (COI) sequence data for a subset of the samples used in my previous microsatellite DNA study (Chapter Three; Addison and Hart 2004) to examine the phylogeographic history of the NW Atlantic populations. I combined traditional genetic analyses (AMOVA, *F*-statistics) and NCA to determine the relative influences of trans-Arctic and trans-Atlantic gene exchange on the genetic diversity of sea urchin populations in the NW Atlantic. I also looked for evidence of a recent population expansion by examining the properties of the mismatch distribution (Rogers and Harpending, 1992; Rogers, 1995).

I used this combination of analyses to test a series of simple biogeographic scenarios. (1) If the last glacial maximum caused the extirpation of sea urchins in the NW Atlantic, followed by a trans-Arctic or a trans-Atlantic migration event, then these geologically young populations should exhibit relatively low haplotype diversity compared to the source population and should have a high frequency of haplotypes that are either identical to or recently descended from haplotypes in the founding population (Zink *et al.*, 2000; Wares and Cunningham, 2001). (2) If NW Atlantic populations are

relatively young, then the mismatch analysis should reveal evidence of recent population expansion. (3) If the Quaternary biogeography of *S. droebachiensis* is similar to some other north Atlantic rocky habitat marine invertebrates, then parsimony and maximum likelihood analyses should produce haplotype networks or phylograms in which NW Atlantic haplotypes are nested within a set of NE Atlantic haplotypes and reflect a strong influence of recent trans-Atlantic gene flow from east to west (Wares 2001; Wares and Cunningham, 2001). (4) Alternatively, if north Atlantic *Strongylocentrotus* biogeography is more strongly influenced by recent trans-Arctic gene flow (Palumbi and Wilson, 1990; Palumbi and Kessing, 1991), then haplotypes in NW Atlantic populations may be derived from (and shared in common with) the older and more diverse Pacific populations.

Materials and methods

Sampling

Samples of *Strongylocentrotus droebachiensis* were obtained as described in Chapter Three (Addison and Hart, 2004). Samples incorporated in this analysis include individuals from the San Juan Islands (Pacific Ocean), Vestifjorden (Norway), Hvalfjordur (Iceland), and six northwest Atlantic populations (Table 4.1). Two specimens identified as *Strongylocentrotus pallidus* collected from the Pacific population were also included as a potential outgroup.

DNA extraction, polymerase chain reaction, and sequencing

Genomic DNA was extracted from ethanol preserved gonad tissue using a standard cetyltrimethylammonium bromide (CTAB) protocol (Grosberg *et al.*, 1996).

Table 4.1 Sampling location (abbreviation), sample size (N), number of segregating sites (S), number of haplotypes observed (H), and diversity measures (standard deviations) calculated using 418 bp of COI.

Population	N	S	H	Haplotype Diversity	Nucleotide Diversity
San Juan Islands, Washington (SJI)	22	23	12	0.7619 (0.0990)	0.0147 (0.0081)
Bonne Bay, Newfoundland (BBY)	7	10	3	0.6667 (0.1598)	0.0087 (0.0057)
Conception Bay, Newfoundland (CBY)	7	24	4	0.8095 (0.1298)	0.0242 (0.0144)
Harve St. Pierre, Quebec (HSP)	7	12	4	0.8095 (0.1298)	0.0096 (0.0062)
Miramichi, New Brunswick (MBY)	21	14	6	0.7619 (0.0688)	0.0120 (0.0068)
East Jeddore, Nova Scotia (JED)	42	17	10	0.7247 (0.0657)	0.0054 (0.0034)
Bear Cove, Nova Scotia (BCV)	48	26	11	0.6702 (0.0725)	0.0091 (0.0052)
Hvalfjordur, Iceland (ICE)	12	3	4	0.5606 (0.1540)	0.0023 (0.0019)
Vestfjorden, Norway (NOR)	15	2	3	0.5905 (0.0771)	0.0016 (0.0015)
Regional Total:					
Pacific	22	23	12	0.7619 (0.0990)	0.0147 (0.0081)
NW Atlantic	132	30	18	0.7163 (0.0390)	0.0093 (0.0052)
NE Atlantic	27	3	4	0.5926 (0.0817)	0.0019 (0.0016)

Polymerase chain reaction (PCR) products for the cytochrome *c* oxidase subunit I (COI) were obtained using the forward primer COIC 5'-TCGTCTGATCCGTCTTTGTCAC-3' and reverse primer COIJ 5'-CAATACCTGTGAGTCCTCCTA-3' described by Edmands *et al.* (1996). These primers amplified a 476 bp section of the COI gene corresponding to base positions 6357-6832 of the *S. purpuratus* genome (Jacobs *et al.,* 1988; Edmands *et al.,* 1996). Amplifications were performed under oil in 12.5 µl volumes containing 10 ng of template DNA, 10 mM Tris-HCL, 50 mM KCl, 0.1% TritonX-100, 0.2 mM of each dNTP (MBI), 2 mM $MgCl_2$ (MBI), 0.3 pmol of each primer, and 0.25 units of *Tsg* Polymerase (Biobasic, Toronto, Canada). The thermal cycling protocol consisted of 37 cycles of 30 sec denaturation at 95°C, 60 sec annealing at 55°C, and 60 sec elongation at 72°C.

Amplified products were visualized in 1.5% agarose (1X TBE) using ethidium bromide. Approximately 75 ng of each product was ethanol precipitated and one strand was sequenced with the COIC primer using Li-Cor IRD-700 Dye Terminators following the manufacture's protocol. The cycle sequencing profile consisted of an initial denaturation at 95°C for 3 minutes followed by 32 cycles of 30 sec at 94°C, 30 sec at 50°C, and 60 sec at 72°C. Excess dye terminators were removed using Sephadex G-50 columns (Sigma) and the products were resolved in 6% (25cm, 0.2mm thick) denaturing polyacrylamide gels using a Li-Cor DNA 4200L-2. Sequences from two *S. pallidus* individuals and 181 *S. droebachiensis* individuals were aligned and edited using the image analysis software provided by Li-Cor.

Statistical analysis

Sequence variation and population structure

Edited sequences were aligned using ClustalX (Thompson *et al.*, 1997) and translated using Gene Jockey. Mean sequence divergence (# of differences) within and among populations was calculated using MEGA version 2.1 (Kumar *et al.*, 2001). Haplotype and nucleotide diversity, analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) and pairwise *F*-statistics were calculated using ARLEQUIN 2.001 (Schneider *et al.*, 2000). For the AMOVA, populations were partitioned into the three separate oceanic regions (Pacific, NW Atlantic, NE Atlantic) that corresponded to the scale of geographic subdivision previously detected using nuclear markers (Chapter Three; Addison and Hart, 2004).

Population growth and range expansion

To determine whether the populations within each oceanic region have recently expanded, mismatch distributions and parameters of the sudden expansion model were estimated using ARLEQUIN. Pairwise differences among sequences within a population are expected to follow a Poisson distribution. Goodness of fit to this model of sudden expansion (Rogers and Harpending, 1992) was estimated using a parametric bootstrap with 10,000 replications (Schneider and Excoffier, 1999). If a population has recently expanded from a small ancestral population with low genetic diversity, then extant lineages should coalesce just prior to the initiation of the expansion (Rogers and Jorde, 1995). Timing of the population expansion can be inferred from the mode of the mismatch distribution (Rogers, 1995). I calculated the timing of population growth using

an estimate of 1 generation per year and sea urchin COI sequence divergence rates of 1.6%-3.5% My^{-1} (Lessios *et al.*, 1999, 2001; McCartney *et al.*, 2000).

Phylogenetic analysis

Neighbour-joining (NJ) trees were constructed for haplotypes within each oceanic region using Kimura two-parameter distances using MEGA. A maximum-likelihood (ML) phylogenetic tree for all unique haplotypes was estimated using PAUP* (Swofford, 2002) with the HKY85+G+I substitution model, TBR branch swapping, and zero branch lengths collapsed. The posterior probability distribution of trees was calculated using Bayesian inference with 10^6 Markov chain Monte Carlo (MCMC) steps using MrBayes Version 3.0 (Huelsenbeck and Ronquist, 2001).

Nested clade analysis

I constructed a 95% parsimony haplotype network using TCS Version 1.13 (Clement *et al.*, 2000). Ambiguous loops in this network were resolved using a combination of coalescent theory (Crandall and Templeton, 1993; Posada and Crandall, 2001; Pfenninger and Posada, 2002) and haplotype relationships defined by the maximum likelihood tree. First, coalescent theory predicts that older haplotypes will be more frequent, and because they are present in populations at a higher frequency they are more likely to originate new haplotypes through mutation events in comparison to younger, less frequent haplotypes. Thus it follows that frequent haplotypes should occur at the interior nodes in the cladogram and have more mutational connections to other haplotypes, and rare haplotypes are expected to be at the cladogram tips with fewer connections (Pfenninger and Posada, 2002). Second, if gene flow is limited among populations then

newly arisen haplotypes are expected to remain close to their evolutionary source. Since dispersal is high among *S. droebachiensis* populations within oceanic regions (Chapter Three; Addison and Hart 2004), the geographic criteria were used to solve loops among haplotypes on this scale. Third, because the sampling design mainly targeted the north Atlantic I likely underestimated the genetic diversity expected within the much older Pacific population. Therefore, the limited frequency and geographic distribution of the Pacific haplotypes precludes the use of coalescent solutions to ambiguous loops involving these haplotypes. To better estimate the haplotype network in these cases I used the maximum likelihood tree topology to resolve the remaining ambiguous loops.

Nesting of the haplotype network was performed following the standard nesting procedures of Templeton *et al.* (1987), Templeton and Sing (1993), and Crandall (1996). NCA was performed using GEODIS Version 2.0 (Posada *et al.*, 2000). Any significant geographic association of haplotypes within each clade was first tested using an exact permutation contingency analysis (10,000 replicates). In order to discriminate between historical (e.g., fragmentation) and contemporary (e.g., restricted gene flow) effects on haplotype frequencies, the latitude and longitude of each population was used to calculate their geographic distances. NCA calculates the clade distance (D_C), which measures the geographical range of a clade, and the nested clade distance (D_N), which describes the geographical distribution of a clade relative to sister clades nested within the group (Templeton *et al.*, 1995). The D_C and D_N values for the interior clades are also compared to the tip clades. The statistical significance of the D_C and D_N values was tested using a random permutation (10,000 replicates) and the outcome was compared to values expected under a randomized null hypothesis of no geographical association (Templeton *et al.*, 1995). For the clades in which the null hypothesis was rejected ($P \leq 0.05$) the

biological cause for the association was inferred using the updated inference key of Templeton (1998; available at http://zoology.byu.edu/Crandall_lab/geodis.htm).

Results

Haplotype diversity

I obtained 418 bp COI sequences (positions 6415-6832 of Jacobs *et al.*, 1988) for all 183 individual sea urchins. There were 32 variable sites and a total of 33 unique haplotypes (GenBank accession numbers: AY504479-AY504511). Mean haplotype and nucleotide diversity were highest for Pacific samples and lowest for NE Atlantic samples (Table 4.1). Sequence divergence was high between the outgroup *S. pallidus* and *S. droebachiensis* (max 5.5%), but was highest between two samples that were identified on the basis of morphological traits (Jensen, 1974; Kozloff, 1987) and microsatellite genotypes as *S. droebachiensis* (5.7%). Overall mean sequence divergence among the haplotypes was 2.2% ± 0.4%.

Population structure

The analysis of molecular variance (AMOVA) indicated a moderate amount of genetic differentiation among the three oceanic regions ($\phi_{CT} = 0.287$, $P = 0.002$). Trans-Arctic pairwise-F_{ST} values were significant and ranged from 0.2139 to 0.3259 (Table 4.2). Larger pairwise values were detected in some trans-Atlantic comparisons (e.g., East Jeddore, Bear Cove, Bonne Bay NFLD), suggesting that there was less gene flow across the Atlantic than across the Arctic. However, pairwise F_{ST} values for other trans-Atlantic

Table 4.2 Pairwise F_{ST} (above diagonal) and their P-values calculated using 1023 permutations of the data (below diagonal). P-values in bold represent significant differences after Bonferroni corrections for multiple tests. Population abbreviations are the same as in Table 4.1.

Region		Pacific	NW Atlantic						NE Atlantic	
	Population	SJI	BBY	CBY	HSP	MBY	JED	BCV	ICE	NOR
Pacific	SJI		0.2656	0.2139	0.2191	0.2364	0.2553	0.2864	0.3259	0.3171
NW Atlantic	BBY	**0.0003**		-0.0333	0.0225	0.0295	-0.0172	-0.0305	0.3945	0.3791
	CBY	**0.0003**	0.6350		-0.0439	-0.0573	0.0136	0.0099	0.1695	0.1925
	HSP	**0.0002**	0.4371	0.7933		-0.0672	-0.0385	0.0057	0.2598	0.2617
	MBY	**<0.0001**	0.2179	0.9024	0.9259		0.0133	0.0376	0.2034	0.2201
	JED	**<0.0001**	0.5473	0.2769	0.7977	0.2209		-0.0050	0.3355	0.3169
	BCV	**<0.0001**	0.6929	0.2956	0.3010	0.0651	0.5367		0.3698	0.3607
NE Atlantic	ICE	**<0.0001**	**0.0004**	0.0250	0.0044	0.0017	**<0.0001**	**<0.0001**		0.0490
	NOR	**<0.0001**	**0.0001**	0.0250	0.0080	**0.0001**	**<0.0001**	**<0.0001**	0.1828	

comparisons were smaller and not significant after Bonferroni correction for multiple tests (Zar, 1999). There was a small but significant amount of genetic differentiation among populations within the NW Atlantic or NE Atlantic oceanic regions ($\phi_{SC} = 0.006$, $P < 0.001$), but tests for pairwise population differentiation (F_{ST}) revealed no significant differences.

Mismatch distribution

The model of sudden expansion was not rejected for any of the three oceanic regions (P-values > 0.05; Table 4.3A). The mismatch distribution for populations in the NE Atlantic is unimodal and reflects the shallow haplotype phylogeny (Figure 4.1). However, the Pacific and NW Atlantic populations show multimodal mismatch distributions and deeper haplotype phylogenies (Figure 4.1). This reflects the low frequency of highly divergent haplotypes (i.e., smaller peaks to the right of the main peak) corresponding to either gene flow from distant populations or introgression of haplotypes between species. Multimodal curves such as these are expected when subdivided populations expand with limited gene flow (e.g., Nm < 20) among regions (Ray *et al.*, 2003). The inferred timing of population expansion is more recent for north Atlantic populations than it is for the Pacific (Table 4.3B).

Phylogenetic analysis

The maximum-likelihood analysis converged on a single tree (Figure 4.2). The deepest divergence between samples distinguished a clade of six haplotypes that included

Table 4.3 **A)** Parameters of the mismatch distribution for populations of *Strongylocentrotus droebachiensis*. Numbers in parentheses are the upper and lower bounds of the 95% CI (1000 bootstrap replicates). *P* is the probability that random mismatch distributions (1000 bootstrap replicates) have larger sums of square deviations than the model of sudden expansion distributions. Mismatch parameters were calculated for all NW Atlantic haplotypes and for a subset of haplotypes that formed a well-supported clade (0.89 posterior probability in Figure 4.2). **B)** Time since population expansion calculated using Tau (95% CI), a generation time of 1 year and a range of COI divergence rates.

A)

Region	Tau	Theta-0	Theta-1	*P*
Pacific	8.500 (3.511-19.532)	5.4380	5.5710	0.611
NW Atlantic (all)	1.500 (0.130-7.567)	2.4060	2.4060	0.612
NW Atlantic (89 Clade)	1.481 (0.218-3,768)	0.0080	1.5260	0.791
NE Atlantic	0.938 (0.281-1.427)	0.0000	725.0000	0.575

B)

	Time since expansion (years)			
	Divergence rate of 1.6%		Divergence rate of 3.5%	
Pacific	635,000	(262,000 - 1,460,000)	290,000	(120,000 - 668,000)
NW Atlantic (all)	112,000	(10,000 – 566,000)	51,000	(4,000 - 259,000)
NW Atlantic (89 clade)	111,000	(16,000 – 282,000)	51,000	(7,000 - 129,000)
NE Atlantic	70,000	(21,000 – 107,000)	32,000	(10,000 - 49,000)

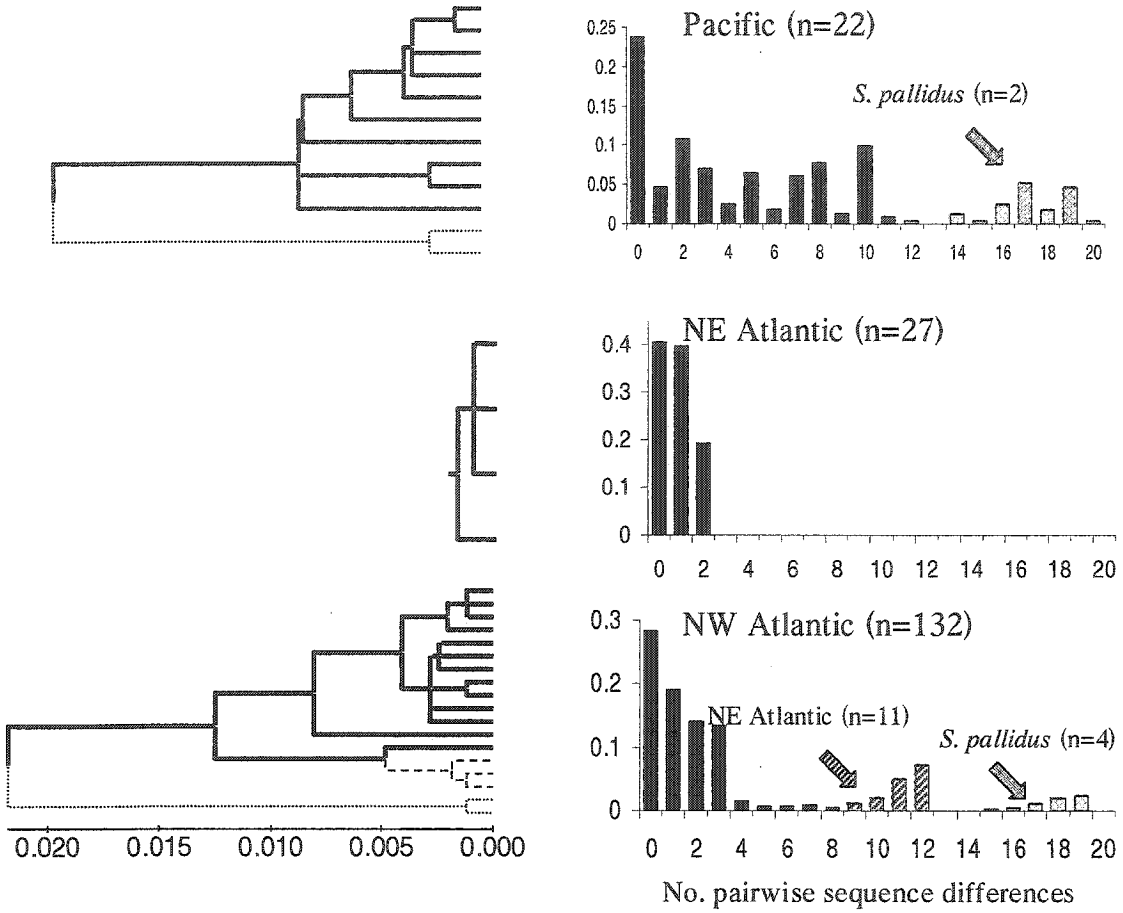

Figure 4.1 The phylogenetic structure of the CO1 haplotypes from *Strongylocentrotus droebachiensis* populations in the Pacific, NE Atlantic and NW Atlantic. For each biogeographic region I show on the left the phylogenic relationships among the haplotypes (linearized neighbour-joining tree, Kimura-2 parameter), and on the right the frequency of the pairwise nucleotide differences (mismatch distribution). The haplotype trees were rooted with the *S. pallidus* sequences (not shown) and are drawn to the same scale. Shading indicates mismatches that are a result of introgression (light grey) and gene flow from neighboring regions (hatched).

both individuals that were identified as *S. pallidus* (haplotypes 32 and 33; Figure 4.2). Four other *pallidus*-like haplotypes (3, 6, 24, 28) came from individuals from Pacific or NW Atlantic populations that had *droebachiensis*-like morphological and microsatellite markers. Hybridization between these two species has been reported in laboratory crosses (Strathmann, 1981).

The remaining *S. droebachiensis* haplotypes did not form monophyletic groups with respect to sampling location: geographically diverse populations shared indistinguishable COI haplotypes in common, and haplotypes collected from both the Pacific and NW Atlantic oceanic regions were widely distributed across the phylogeny. The majority of the haplotype diversity in the NW Atlantic was descended from a single Pacific ancestor (Figs. 4.2, 4.3), and the shared haplotype (1) was widespread among NW Atlantic populations. This result suggests a major colonization event from the Pacific to the NW Atlantic that is still evident in contemporary populations. However, the low posterior probabilities for NW Atlantic clades including haplotypes 22 and 27, and the large distances between these haplotypes and their common ancestors, suggest that these haplotypes are relicts of past colonization events. Furthermore, similar microsatellite allele frequencies across the Arctic (Chapter Three; Addison and Hart 2004) indicate that the history of connection between the Pacific and the NW Atlantic is one of either repeated gene exchange or massive historical migration followed by little genetic drift in NW Atlantic populations. Evidence for population expansion in the NW Atlantic comes mainly from one well-supported clade (called the 89 calde; Table 4.3A; Figure 4.2) of 10 haplotypes. Inclusion of the highly divergent haplotypes (14, 22, 23, 26, 27) does not affect estimated coalescent times for the NW Atlantic haplotypes (Table 4.3B), but does obsecure the pattern of population expansion (Theta-0 versus Theta-1, Table 4.3A). The

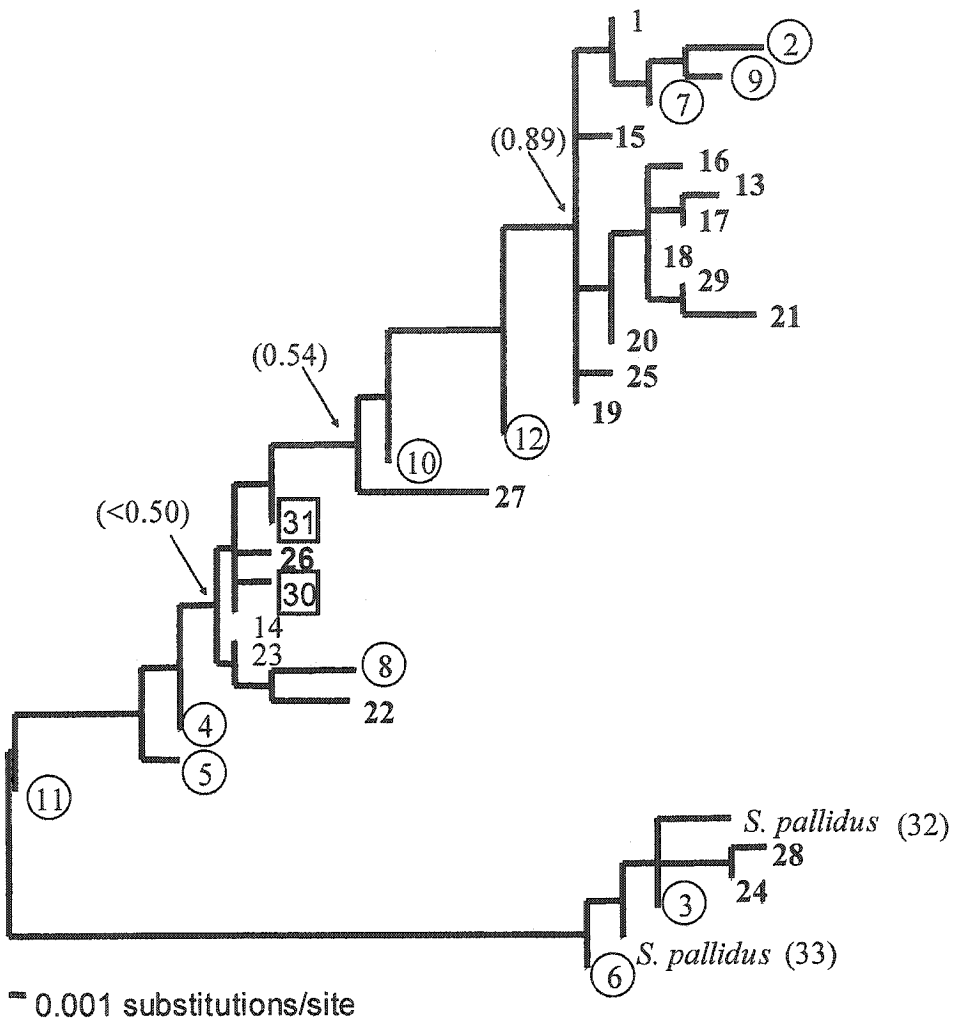

Figure 4.2 Maximum-likelihood tree of the 33 *Strongylocentrotus* COI haplotypes, with the posterior probability for three clades indicated in parenthesis. Unique haplotypes are indicated by their numbers. Those haplotypes found only in the Pacific Ocean are outlined with circles, NW Atlantic in boldface, NE Atlantic outlined with squares, and those shared between oceans are in plain text. Outgroup sequences are *S. pallidus*.

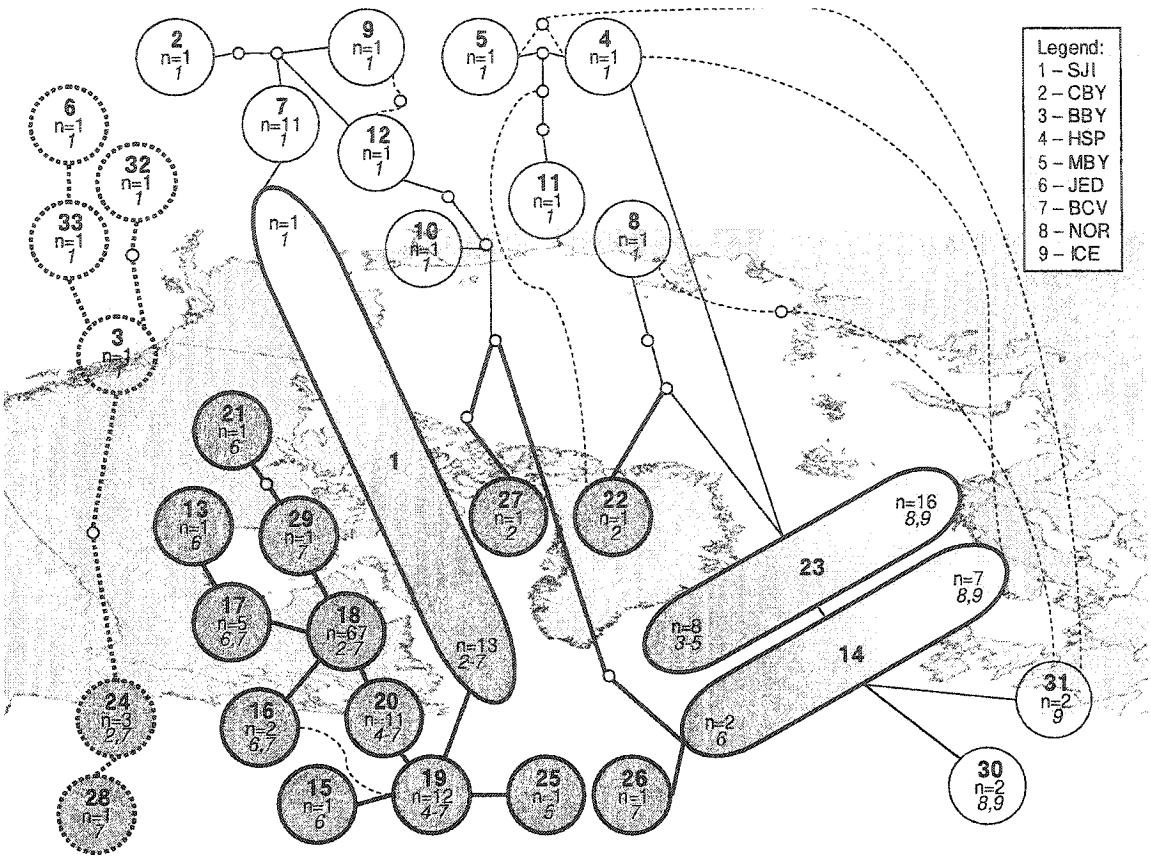

Figure 4.3 95% plausible networks for *Strongylocentrotus* COI haplotypes. Haplotyes found in the NW Atlantic are shaded in grey and haplotypes shared among oceanic regions are represented by ovals. Haplotype numbers are identified in boldface, n is the haplotype sample size and numbers in italics indicate the population in which the haplotypes were detected (for detailed resolution of NW Atlantic populations see Figure 3.1). Lines connect haplotypes that differ by one mutation; small open circles indicate missing intermediate haplotypes. Thin dashed lines represent ambiguous loops that were resolved using coalescent and ML methods (see results). Samples of *S. pallidus* (32, 33) and four haplotypes from *S. droebachiensis* form a separate network in the Pacific and NW Atlantic which is indicated by heavy dotted lines.

contrast between these two results suggests that population expansion within the NW Atlantic and gene flow into the NW Atlantic happened at the same time.

Haplotypes in the NE Atlantic were descended from a different Pacific ancestral sequence than those in the NW Atlantic, but the large sequence divergence between Pacific and NE Atlantic haplotypes prevented me from reliably linking the NE Atlantic haplotypes to the rest of the TCS network (Figure 4.3) and left relationships between NE Atlantic and other haplotypes largely unresolved in the ML phylogram (Figure 4.2). For example, four Pacific haplotypes (4, 5, 8, 11) can be related to haplotypes common in the NE Atlantic (14, 23), but these four haplotypes are so divergent from other Pacific haplotypes that the TCS algorithm linked them (and several others) by a relatively large number of unobserved intermediates to other Pacific haplotypes via a NW Atlantic haplotype (27; Figures 4.2, 4.3). This linkage is biogeographically unrealistic, and probably reflects my undersampling of a much greater haplotype diversity within Pacific populations.

Haplotype diversity was lower in the NE Atlantic than in the NW Atlantic. Three of the four NE Atlantic haplotypes were found in both Norway and Iceland, and two of these haplotypes were also found at low frequency in the NW Atlantic (Figure 4.3). One shared haplotype (23) was only found in western Newfoundland and the Gulf of St. Lawrence, and the other (14) was only found in Nova Scotia. There was one unique haplotype in the NW Atlantic (26) that was descended from a NE Atlantic ancestor. The widespread occurrence of these shared haplotypes in NW Atlantic populations suggests large scale trans-Atlantic gene flow into these populations.

Nested clade analysis

The statistical parsimony analysis yielded two networks. Network 1 (n = 175) contained 27 *S. droebachiensis* haplotypes and Network 2 (n = 8) contained the two haplotypes from *S. pallidus* (32, 33) and four from *S. droebachiensis* (3, 6, 24, 28) (Figure 4.3). The number of changes that connect the two networks exceeds that supported by the 95% statistical parsimony analysis. Due to the limited sample size of Network 2, I restricted my analysis to the 27 haplotypes in Network 1. The 95% plausible set of network connections contains several ambiguous connections, many of which are likely a result of the deep but under-sampled ancestral polymorphism within the Pacific population. Connections between haplotypes 18/16 and 4/23 were resolved based on the frequency and topology criteria. Connections between haplotypes within ocean regions were favoured over those between ocean regions, and two separate loops involving unobserved intermediate haplotypes in the Pacific were resolved using the maximum likelihood tree topology. The nesting design of this final network is given in Figure 4.4.

The categorical analyses of geographic association within each clade revealed significant genetic structure in six of the nested haplotype groups (Table 4.4). Further resolution of these patterns was obtained when the geographic distances were included (Table 4.4). There are strong inferences of range expansion across the north Atlantic and into the Pacific (Clades 1-1, 2-1, 3-1). The frequency and distribution of haplotypes 14 and 23 in clades 1-1 and 2-1 indicate that the range expansion across the Atlantic was from the east to west. A similar pattern exists in clade 3-1, but the inference of contiguous range expansion is from the NE Atlantic into the Pacific. This pattern is opposite to expectations based on other biogeographical patterns (Vermeij, 1991), but

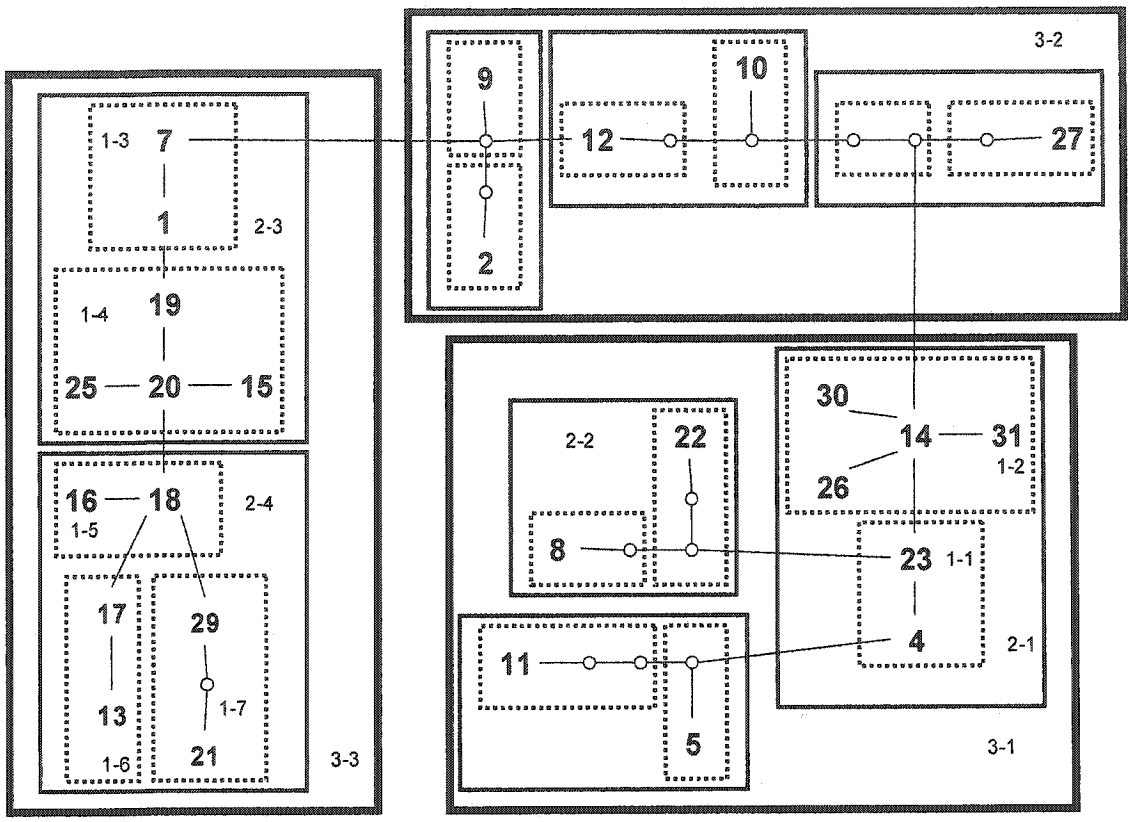

Figure 4.4 The nested clades for the resolved haplotype Network 1 of *Strongylocentrotus droebachiensis* COI sequences. Each line represents a single mutation and the small open circles represent haplotypes not observed. Numbers represent the unique haplotypes and are the same as in Figs. 4.2 and 4.3. Dotted lines delineate one-step clades, thin solid lines two step clades, and heavier solid lines three-step clades. Clade numbers (e.g., 1-1) are indicated for clades in which a contingency analysis of geographical association among haplotypes was possible.

Table 4.4 Nested clade analysis results for *Strongylocentrotus droebachiensis*. *P*- values are for the contingency analysis of geographical associations of haplotypes and were obtained by permuting the data 10,000 times. Inferences of contiguous range expansion (CRE), isolation by distance (IbD), restricted geneflow (RGF), and long distance dispersal (LDD) were made using the updated version of Templeton's (1998) key. (*ns – no significant clade differences, § Pattern is confounded by ancestral polymorphism, *see text.*)

Clade	Chi-Square *P*-value	Chain of Inference	Outcome
1-1	0.0821	2-11-12-N	CRE from NE to the NW Atlantic *and Pacific* §
1-2	**0.0148**	*ns	
1-3	**<0.0000**	2-11-12-13-14-N	CRE or IBD from Pacific into NW Atlantic
1-4	0.9038	*ns	
1-5	1.0000	*ns	
1-6	0.3336	*ns	
1-7	1.0000	2-11-17-4-N	RGF with IbD between two NW Atlantic populations
2-1	0.0859	2-11-12-N	CRE from NE to NW Atlantic *and Pacific* §
2-2	1.0000	2-11-17-N	Inconclusive
2-3	**<0.0000**	2-3-4-N	RGF with IbD between the Pacific and NW Atlantic
2-4	0.9367	*ns	
3-1	**0.0087**	2-11-12-N	CRE from NE to NW Atlantic *and Pacific* §
3-2	0.1986	2-11-17-N	Inconclusive
3-3	**0.0002**	2-3-4-N	RGF with IbD between the Pacific and NW Atlantic
total cladogram	**<0.0000**	2-3-5-6-7-8-N	IBD or LDD from Pacific into north Atlantic

could simply be a result of the poor resolution of the haplotype network owing to the high ancestral polymorphism in the Pacific (see above). When I removed the Pacific haplotypes from the analysis the results were still consistent with a pattern of contiguous range expansion across the north Atlantic (data not shown).

Populations in the NW Atlantic share a different history with the Pacific. At the lowest clade level (1-3) a trans-Arctic migration event occurred from the Pacific into the NW Atlantic, but my sampling scheme was inadequate to determine whether this event was a result of contiguous range expansion, long distance colonization, or past fragmentation (Table 4.4). Higher-level clades (2-3 and 3-3) reveal patterns of restricted gene flow and isolation by distance between the Pacific and the NW Atlantic. Although a significant geographic pattern existed among the haplotypes at the highest nesting level (total cladogram), the sampling scheme was inadequate to discriminate between isolation by distance (short distance movements) and long distance dispersal.

Discussion

Phylogeographic history

Despite first arriving in the NW Atlantic 3.5-3 Mya (Vermeij, 1991), the current population genetic structure of *Strongylocentrotus droebachiensis* was shaped by recent trans-Arctic and trans-Atlantic migration events. The dispersal of long-lived planktonic larvae has resulted in regional panmixia without local differentiation at either nuclear (Chapter Three; Addison and Hart, 2004) or mitochondrial loci. However, the analysis of molecular variance (AMOVA) revealed a significant genetic discontinuity among the three oceanic regions ($\phi_{CT} = 0.287$, $P = 0.002$). This result is consistent with my previous

analysis using microsatellite DNA (Chapter Three; Addison and Hart, 2004), although the patterns of haplotype diversity and genetic subdivision offer better resolution of historical processes affecting these populations. In light of the known glacial history of the Arctic and north Atlantic, the polyphyletic nature of the Pacific and Atlantic COI haplotypes indicates that a history of sporadic migration has prevented any complete isolation of populations throughout the species range. The small amount of divergence among regions and widespread sharing of identical haplotypes suggests that the populations on both coasts of the north Atlantic share a long, and independent, history of colonization from the Pacific.

The NCA provided a robust basis for interpreting the evolutionary history of the populations. The main patterns revealed in this analysis were (1) the restricted gene flow and isolation by distance of haplotypes from the Pacific into the NW Atlantic and (2) the westerly range expansion across the north Atlantic. The coalescent approach used by NCA is able to further resolve the timelines of these events by assuming that processes in the lower clade levels are more recent (Templeton *et al.*, 1995; Templeton, 1998). However, the interpretation of a very recent trans-Arctic migration event in clade 1-3 seems unlikely because this clade is internal to the rest of the NW Atlantic lineage. At higher clade levels (at which clade 1-3 is now internal) NCA indicates that a trans-Arctic migration event occurred followed by a history of isolation by distance.

The majority of the diversity in the NW Atlantic is descended from a single Pacific haplotype (1), and these findings are consistent with the pattern described by Palumbi and Wilson (1990). This similarity to previous results is striking because I increased the sampling of individuals within the NW Atlantic more than six-fold and increased my sampling of nucleotide sites using sequences (rather than RFLPs). Because

I only detected one shared haplotype between the two regions, it is unlikely that gene exchange is continuous. However, previous results using microsatellite DNA indicate that the magnitude of gene exchange across the Arctic was large enough to produce broad allelic similarity between the two oceans, with small pairwise F_{ST} values between Pacific and NW Atlantic populations of 0.019 to 0.032 (based on data in Chapter Three; Addison and Hart, 2004). One possible explanation for this observation is that lineage sorting has resulted in the complete loss of all but three ancestral mtDNA haplotypes in the NW Atlantic, of which one and its descendents now dominate the NW Atlantic. However, I may have failed to detect other shared haplotypes because of my limited sampling in the Pacific. Assuming that no other more recent trans-Arctic migration events have occurred, COI divergence rates calculated for sea urchins between 1.6% and 3.5% My^{-1} (McCartney *et al.*, 1999; Lessios *et al.*, 1999, 2001) indicate that the migration event occurred between 0.90 and 0.41 Mya (1.4% divergence between haplotypes 1 and 21, Figure 4.3). This timeline is slightly older than the previous estimation of migration 0.3 Mya (Palumbi and Wilson, 1990), and both are consistent with gene flow during the interglacial periods in the late Pleistocene. In any case, these results are not consistent with a hypothesis of local extirpation followed by recent recolonization from the NE Atlantic and suggest instead the persistence of *S. droebachiensis* in glacial refugia in the NW Atlantic during more recent glacial cycles (see Holder *et al.*, 1999).

NCA inferred a range expansion across the north Atlantic, but patterns of haplotype diversity and phylogenetic relationships indicate that populations in the NW Atlantic were not founded by recent NE Atlantic colonizers. Two of the haplotypes detected in the NE Atlantic are found at very low frequencies in the NW Atlantic, and I only found one unique haplotype in the NW Atlantic that was descended from a NE

Atlantic ancestor by a mutation event following gene flow from east to west. Wares and Cunningham (2001) found evidence of local extirpation and trans-Atlantic recolonization in some NW Atlantic species, in contrast with evidence for persistence through Pleistocene glacial cycles in other species that must have survived in refugial populations in the NW Atlantic. My results for *S. droebachiensis* are more similar to the latter patterns. If the patterns of range expansion are assumed to be the most recent events detected for sea urchins in the NW Atlantic, then the timing of population growth obtained from the mismatch distribution indicates that these trans-Atlantic dispersal events could have happened since the last glacial maximum (0.02 Mya).

Although natural dispersal of planktonic larvae in the western Greenland and Labrador currents could explain the presence of NE Atlantic COI haplotypes in the NW Atlantic, human mediated dispersal is also a possible explanation. Passage via the fouling community or ballast water of ships trading saltwater fish and coal across the north Atlantic has been suggested for a red alga (*Phycodrys rubens*; van Oppen *et al.*, 1995). Similar human-aided translocation of the brittle star *Ophiactis savignyi* has been documented between the Pacific and Western Atlantic (Roy and Sponer, 2002).

Genetic exchange between the Pacific and NE Atlantic appears to be low. Both microsatellite allele diversity (Chapter Three; Addison and Hart, 2004) and COI haplotype diversity are lower in NE Atlantic populations of sea urchins. These results are consistent with either a recent founding event or a long history of isolation. Evidence for the former hypothesis is weak because founding haplotypes are expected to be deeply nested and higher in frequency in the founding population (Castelloe and Templeton, 1994; Wares, 2001), a pattern not observed in my data. It is clear that finer resolution of the historic relationship among NE Atlantic and Pacific sea urchins requires more

sampling throughout the Canadian Arctic archipelago, Scandinavia, Russia, and Greenland. Migratory routes for dispersing planktonic larvae in the NE Atlantic are likely along the western Greenland Current to Baffin Island where the southern flow of the Labrador Current could carry sea urchins from the Arctic and Pacific Oceans to the NW Atlantic coast. Such sampling would also help to clarify the striking differences between sea urchin populations in the northeast Pacific and the northwest Atlantic: are they merely isolated by distance (and by a genetic cline across the Canadian Arctic), or is there a real barrier to dispersal across the Arctic?

The Arctic Ocean as a barrier to dispersal

The patterns of local genetic homogeneity among *S. droebachiensis* populations with a significant haplotype discontinuity across a biogeographic barrier are consistent with other population genetic studies of sea urchins with dispersing planktonic larvae. Temporary barriers to dispersal caused by sea level changes likely caused significant genetic differentiation among several species of tropical sea urchins in the Indo-West Pacific (*Diademia*; Lessios *et al.*, 2001; *Eucidaris;* Lessios *et al.*, 1999). This reflection of historic dispersal barriers is also evident in many other studies of marine invertebrates with an Indo-West Pacific range (Benzie, 1999). The temporally variable dispersal barriers across the Arctic and north Atlantic formed during the Pleistocene glaciations have had a similar effect on the population genetics *S. droebachiensis*. Other global barriers to dispersal detected in sea urchins include the Isthmus of Panama, the long stretches of ocean diving both the Atlantic and Pacific, cold water upwelling off the tip of South Africa, and the freshwater plume of the Orinoco and Amazon rivers (Lessios *et al.*, 2003).

Several studies of sea urchins suggest that population genetic patterns are often discordant among species with similar larval dispersal abilities. One interpretation of these patterns is that some biogeographic barriers are differentially porous to dispersal by larvae of different species. For example, gene flow is limited between Caribbean and Brazilian populations of the sea urchins *Tripneustes* (Lessios *et al.*, 2003), *Diadema* (Lessios *et al.*, 2001) and *Echinometra* (McCartney *et al.*, 2000), but not of *Eucidaris* (Lessios *et al.*, 1999). Across the Indo-Pacific ranges of these genera, *Tripneustes* species show genetic homogeneity at this scale, but *Diadema* and *Eucidaris* do not. The discordant population genetic patterns shown by species with similar larval dispersal ability suggest that population genetic structure across a broad geographic range is likely the result of the stochastic nature of larval dispersal and colonization (Palumbi, 1996; McCartney *et al.*, 2000), and that patterns of allele and haplotype frequency variation may be difficult to interpret clearly in terms of contemporary gene flow alone.

Genetic studies of marine invertebrates with circum-Arctic ranges also suggest that both trans-Arctic and trans-Atlantic dispersal is sporadic but effective. For example, phylogenetic analyses of the *Macoma balthica* and *Mytulis edulis* species complexes reveal strikingly similar histories of recent trans-Arctic contact between populations (Rawson and Hilbish, 1995; Väinölä, 2003). The divergence between members of both of these taxa began soon after the original introduction of *Macoma* and *Mytilus* to the North Atlantic following the opening of the Bering Strait. Their population genetic patterns have been interpreted in the context of repeated invasions of the Atlantic by larval dispersal from Pacific populations, which resulted in secondary contact between diverged Pacific and Atlantic lineages on both Atlantic coasts. Repeated trans-Arctic dispersal has also been described for other organisms including marine algae (van Oppen *et al.*, 1995).

However, in other species the trans-Arctic colonization events were thought to have occured prior to the major glaciation events of the Middle Pleistocene (Reid, 1990; Zaslavskys *et al.,* 1992; Taylor and Dodson, 1994).

The North Atlantic as a barrier to dispersal

The previous comparative studies by Wares and Cunningham (2001) of trans-Atlantic mtDNA phylogeography revealed that the NW Atlantic rocky substratum communities include a mixture of species that were locally extirpated and re-established by recent range expansion from glacial refuges in Europe and other species that must have survived with relatively large effective population sizes in NW Atlantic refuges. Other analyses have expanded the list of species that probably persisted in the NW Atlantic in refuges (F. Harper, unpublished data). Westheide *et al.,* (2003) suggest that trans-Atlantic dispersal by polychaete larvae might be frequent and effective. My results suggest that the views of both Palumbi and Wilson (1990) and Wares and Cunningham (2001) are essentially correct. Recent trans-Atlantic dispersal of sea urchin larvae has effectively distributed mtDNA haplotypes from Europe to North America (Wares and Cunningham, 2001), but in the context of historical colonization from the Pacific (Palumbi and Wilson, 1990) and survival of those original NW Atlantic populations during the Quaternary glaciations, trans-Atlantic gene flow has had a relatively minor influence on both nuclear (Addison and Hart, 2004) and mitochondrial population genetic structure (Figure 4.1).

Combining nuclear and mitochondrial markers

One useful outcome of the combination of mtDNA haplotypes and nuclear microsatellite allelic markers in this study was the ability to identify the striking contribution of introgression to haplotype variation. Conspecific hybridization with *S. pallidus* was detected throughout the range of *S. droebachiensis* in the NW Atlantic and Pacific. However, the overall contribution to haplotypic diversity in the NW Atlantic was small (two haplotypes in four individuals). The patterns of hybridization detected in this study are not the patterns that would be predicted from the results of Strathmann (1981). In controlled laboratory crosses, Strathmann (1981) determined that the ova of *S. droebachiensis* were readily fertilized by the sperm of *S. pallidus*, but fertilization rates were very low in the reciprocal cross. I found *S. pallidus* mtDNA haplotypes in individuals morphologically identified as *S. droebachiensis*, a pattern that is only likely to be produced when *S. pallidus* eggs are fertilized by *S. droebachiensis* sperm. These same individuals had *droebachiensis*-like microsatellite genotypes. The two species' nuclear genomes could be qualitatively distinguished because the microsatellites described in Chapter Two (Addison and Hart, 2002) failed to amplify in any known *S. pallidus* samples that I tested (suggesting that *S. pallidus* populations are fixed for null alleles at these microsatellite loci). The combination of *pallidus*-like maternal markers and fully *droebachiensis*-like nuclear and morphological markers suggests that these four individuals were descendants of female hybrids by one or more generations of matrilineal back-crossing with male *S. droebachiensis* (and not male *S. pallidus*). Such differential back-crossing would be consistent with Strathmann's (1981) hybridization results.

Second, the documentation of significant population genetic effects of hybridization and back-crossing suggests an additional mechanism affecting my previous

measures of microsatellite allele and genotype frequencies. I found large departures from HWE and deficits of heterozygotes at most loci in most populations (Chapter Three; Addison and Hart, 2004). Because I found no null homozygotes in NW Atlantic populations, I concluded that the overall frequency of null alleles in those populations must be low. However, the low frequency of *S. pallidus* introgression observed in the *S. droebachiensis* haplotype data suggests that some part of the heterozygote deficits I detected at nuclear loci in *S. droebachiensis* could be due to the segregation of *S. pallidus* null alleles via hybridization.

Third, the combination of haplotypes and microsatellites for the same set of sea urchin populations allows me to put the measures of microsatellite population differentiation into an independent context. The biological significance of allozyme or microsatellite F_{ST} estimates that are numerically small but statistically significantly different from zero is sometimes questionable. Because the microsatellite loci have been used only in *S. droebachiensis*, the microsatellite results cannot be put into the context of previous allozyme studies of other sea urchins. However, measures of population differentiation based on COI haplotypes can be put into the more general context of previous sea urchin studies using mitochondrial protein-coding genes. For example, Edmands *et al.* (1996) used COI haplotypes to measure population differentiation as F_{RT} = 0.064 between groups of *S. purpuratus* north and south of Los Angeles, California; the corresponding F statistic based on allozymes was small and not significantly different from zero. I found pairwise F_{ST} values of 0.21-0.28 based on COI haplotype comparisons between Pacific and NW Atlantic *S. droebachiensis* populations (Table 4.2); the corresponding measure of microsatellite differentiation among 11 Atlantic and Pacific populations was F_{ST} = 0.087 (Chapter Three; Addison and Hart, 2004). The significant

haplotype differentiation in *S. droebachiensis* (comparable to haplotype differentiation detected in other sea urchins using homologous sequences) suggests that my previous estimates of numerically small levels of population differentiation using microsatellites may be biologically highly significant.

CHAPTER FIVE

TEMPORAL GENETIC STRUCTURE IN SEA URCHINS: EVIDENCE FOR SWEEPSTAKES REPRODUCTION.

Introduction

Large variance in reproductive success is a predicted consequence of the high dispersal life history syndrome common to many marine invertebrates (Hedgecock, 1994). Large census population sizes, high fecundity, broadcast spawning with external fertilization, and long lived planktonic larvae with the potential for extensive dispersal make many marine species susceptible to reproductive failure. This can be attributed to the unpredictability of sperm and egg interactions in the plankton (e.g., Levitan *et al.*, 1992; Levitan, 2002), potential for sperm limitation (Yund, 2000), food limitation for planktotrophic species (Olson and Olson, 1989), mortality from predators (Rumrill, 1990) and other predictable or stochastic features of the marine environment (e.g., Fogarty *et al.*, 1991). If variance in reproductive success frequently reduces the effective number of breeders that contribute to each pool of offspring due to such features of the reproductive environment, then reproduction in free spawning marine invertebrates can be viewed in large part as a "sweepstakes" in which an individual's success during any one reproductive event is largely independent of its genotype.

Variance in reproductive success influences genetic diversity by reducing effective population size (N_e) and increasing susceptibility to random genetic drift. This may partially explain the more than 10^2- to 10^5-fold reduction in the estimates of N_e

relative to the census population sizes of many marine species (e.g., Avise *et al.*, 1988; Palumbi and Wilson, 1990; Hedgecock, 1994; Avise, 2000; Turner *et al.*, 2002). Small N_e also influences the genetic composition of different cohorts of marine larvae. First, because only a subset of adults are expected to contribute to each reproductive event, cohorts of offspring are expected to exhibit reduced genetic diversity relative to spawning adult populations (Hedgecock, 1994). Second, because a different subset of adults is expected to be successful in different spawning events, random sampling error should result in cohorts that are genetically differentiated over time (Li and Hedgecock, 1998).

Hedgecock's (1994) sweepstakes hypothesis provides a potential explanation for the patterns of 'chaotic' genetic patchiness frequently observed in population genetic studies of marine invertebrates with the high dispersal life history syndrome (eg. Johnson and Black 1984a; Burnett *et al.*, 1994; David *et al.*, 1997; Johnson *et al.*, 2001; Addison and Hart, 2004). In these cases populations were genetically homogeneous over a large spatial scale, but demonstrated some statistically significant small-scale genetic heterogeneity. While similar allele frequencies throughout the range of these species are consistent with high gene flow facilitated by dispersing larvae, the small scale heterogeneity requires either differential post-recruitment mortality or temporal variation in the composition of new recruits (Hedgecock, 1994). Several empirical studies in marine species have revealed striking temporal variation between adults and new recruits sampled at the same sites (Johnson and Black, 1982, 1984b; Watts *et al.*, 1990; Johnson *et al.*, 1993; Edmands *et al.*,. 1996; Johnson and Wernham, 1999; Lundy *et al.*, 2000; Moberg and Burton, 2000; Planes and Lenfant, 2002), and these patterns are consistent with the among-cohort genetic differentiation predicted by sweepstakes reproduction (Li and Hedgecock, 1998).

If cohorts of larvae are genetically differentiated, then age structured populations should demonstrate unrecognized temporal genetic structure resulting in heterozygote deficiencies as an expression of the Wahlund effect (Watts *et al.,* 1990; Borsa *et al.,* 1991; Edmands *et al.,*, 1996; David *et al.,* 1997; Lewis *et al.,* 2000). For example, in an allozyme analysis of the bivalve *Ruditapes decussates*, Borsa *et al.* (1991) detected no heterozygote deficits in two cohorts of newly-settled juveniles and one single age adult cohort, but they detected significant heterozygote deficiencies in multi-cohort samples of adults. Several explanations exist for the frequent observations of heterozygote deficits in bivalves and other marine invertebrates (e.g., Zouros and Foltz, 1984). Although many authors suggest that the temporal variance in allele frequencies among cohorts arises due to spatially and temporally varying selection on larvae (e.g., Johnson and Black, 1984a; David *et al.,* 1997), an alternate explanation is sampling variance caused by the sweepstakes reproductive success of a small fraction of the population (Hedgecock, 1994).

Sea urchins in the genus *Strongylocentrotus* have widely dispersing planktonic larvae, adults are long-lived (Ebert, 1967, Robinson and MacIntyre, 1997; Meidel and Scheibling, 1998; Ebert *et al.,* 1999; Vadas *et al.,* 2002), and their population dynamics are influenced by sporadic and unpredictable recruitment (Ebert *et al.,* 1994; Harris *et al.,* 1994; Miller and Emlet, 1997; Balch *et al.,* 1998; Balch and Scheibling 2000). These life history characteristics make *Strongylocentrotus* susceptible to large variance in reproductive success. Population genetic analyses of age-structured populations of *S. purpuratus* and *S. franciscanus* on the west coast of North America have revealed patterns consistent with sweepstakes reproduction (Edmands *et al.*, 1996; Moberg and Burton, 2000). Both of these allozyme studies revealed genetic patchiness and significant

levels of genetic differentiation among populations of adults and recruits. Furthermore, Edmands *et al.* (1996) also found significant heterozygote deficiencies in larger size classes of *S. purpuratus*, a pattern which they interpreted as a temporal Wahlund effect driven by the accumulation of genetically different cohorts. However, in a more detailed study using maternally inherited mtDNA (COI) haplotypes, Flowers *et al.* (2002) failed to support the sweepstakes hypothesis in *S. purpuratus*. The additional sampling of haplotype frequencies in their study revealed no substantial and consistent temporal or spatial genetic variation either among cohorts of new recruits, or between cohorts of new recruits and adult populations. Flowers *et al.* (2002) concluded that females do not experience a large variance in reproductive success proposed by the sweepstakes hypothesis.

Strongylocentrotus droebachiensis populations in the northwest Atlantic also show genetic patterns of variation among populations that are consistent with sweepstakes reproduction (Addison and Hart, 2004). Here I extend these analyses to include age-class data for populations of adults for which I have both microsatellite and COI haplotype frequency data. I also include new analyses of microsatellite allele frequencies for a cohort of planktonic larvae and a cohort of newly settled recruits. I evaluate whether different year classes are genetically differentiated and whether the large heterozygote deficits ($F_{IS} = 0.1436$, $P < 0.05$) observed in the microsatellite data are explained by the accumulation of genetically different cohorts. By including both nuclear and mitochondrial DNA markers, this analysis may improve the resolution of any sex biased variance in reproductive success. Neither the microsatellite (Chapter Three; Addison and Hart, 2004) nor the mtDNA haplotype (Chapter Four) data revealed any significant genetic structure in the northwest Atlantic ($F_{ST} = 0.0016$, $P > 0.05$, and $\Phi_{ST} =$

0.0369, $P = 0.0543$, respectively), but there was some small scale heterogeneity observed in the allelic frequencies of both markers. If sweepstakes reproduction is a general feature of *S. droebachiensis,* then this small scale genetic heterogeneity may be a result of significant temporal and spatial genetic differentiation among the year classes. If the significant deficit in heterozygotes observed in the multiage adult population are explained by the accumulation of genetically differentiated cohorts over time, then individual cohorts are not expected to demonstrate consistent heterozygote deficits until they are pooled at the population level.

Materials and Methods

Collection

Adult sea urchins were collected in the summer of 1999 using SCUBA from Miramichi Bay, Main-a-Dieu, East Jeddore, Bear Cove, and Digby (Table 3.1). Shallow water populations of sea urchins (< 20 m) in Bear Cove and East Jeddore experienced near complete mortality in 1995 as a result of an epidemic outbreak of the parasitic amoeba, *Paramoeba invadens* (Scheibling and Hennigar, 1997). Samples from these populations were included in the analysis because planktonic recruitment may be important in the recovery of populations following disease epidemics. Such populations are expected to consist mostly of a limited number of age classes that are younger than the epidemic. The analysis of haplotype and allele frequency variation among age classes in such populations may be clearer than age structure analysis in populations that are strongly influenced by recruitment and by post-recruitment migration of adults. The sample of newly metamorphosed recruits provided by T. Balch was collected at Isle of Shoals (42° 97' N, 76° 62' W) over several weeks in the spring of 1994 using an artificial

settlement substrate (Balch *et al.*, 1998; Balch, 1999). Planktonic larvae (4-8 arm stage) were collected from surface waters of Halifax Harbour (43° 39' N and 63° 34' W) on a single day in October 2002 using a vertically towed plankton net.

For clarity, I refer to *cohorts* as samples assigned to the same age class that are collected at the same geographic location. *Year classes* are samples assigned to the same settlement year, regardless of sampling location, and *populations* are samples collected at the same location, regardless of age.

Measurement and aging

Live adults were processed within one week of collection. Measurements of horizontal test diameter were taken using vernier calipers (0.1 mm accuracy) and the Aristotle's lantern was dissected and stored at -80 C for aging. Sea urchins were aged following Robinson and MacIntyre's (1997) modification of Jensen's (1969) technique, which uses annual growth increments in a calcium carbonate skeletal spicule (the rotule) to estimate years since metamorphosis. All five rotules were dissected from each Aristotle's lantern, charred over an alcohol flame, embedded in a mounting resin (Crystalbond™ 509, Aremco Products, Inc., New York, USA), and sanded along the longitudinal plane using fine grit sandpaper. Both the light and dark rings corresponding to periods of fast (summer) and slow (winter) growth, respectively (Jensen, 1969), were viewed using a dissecting microscope. Paired light and dark rings represent one year of growth and were used to determine the age of each sea urchin. Ages were estimated from two or more rotules from each individual to confirm consistent age assignment within individuals. These ages were then used to determine the year that each individual settled in its respective population.

Natural growth lines in the ossicles of sea urchins have been recognized for over a century (see Gage and Tyler, 1985) but the validation of the annual addition of growth lines has recently been criticized. Russell and Meredith (2000) tagged sea urchins with a fluorescent marker (tetracycline) that is incorporated into the growing carbonate ossicles, released the sea urchins into a tide pool, and collected them one year later. In 23% of individual sea urchins, no clear growth line was produced adjacent to the tetracycline mark or more than one growth line was produced. However, because food abundance, temperature, and other environmental conditions in tide pools vary on time scales shorter than one year, growth rates of sea urchins in these habitats are likely to be highly variable within years, and sea urchins in such habitats might be less likely to produce annual growth lines. Other field studies focusing on subtidal populations of sea urchins (such as those collected in my study) have validated growth lines as chronometers (Vadas and Beal, 1999; Vavrinec *et al.*, 2001). Thus, the ages reported for sea urchins in my study are based on reliable aging techniques. Only the age assignments for the oldest individuals (>15 years) are likely to be problematic because overall growth rate declines with age, the growth increment between annual rings is smaller, and the annual rings are more difficult to distinguish. However, any mistakes in age assignments are more likely to make cohorts genetically more similar to each other. Significant differentiation among the age classes reported here suggests that any mistakes in aging older adults had a limited influence on the population genetic comparisons.

Genetic analysis of age classes

Four-locus microsatellite genotypes for adult sea urchins were previously reported for sea urchins collected from Miramichi Bay, Main-a-Dieu, East Jeddore, Bear Cove,

and Digby (Chapter Three; Addison and Hart, 2004). Following the age analysis, individual genotypes from each population were sorted by their settlement year. I present analyses for the 2-4 most abundant age classes (represented by 9 or more individuals) from each population. Although many pairs of populations had some individuals of the same age, these most-abundant year classes were shared in common between populations in only four cases (Miramichi Bay and Main-a-Dieu in 1987; Miramichi Bay and Jeddore in 1989 and 1990; Digby and Isle of Shoals in 1994). Similarly, the 418 bp of cytochrome *c* oxidase subunit I (COI) sequence data reported for individuals in Chapter Four were also sorted with respect to settlement location and year (Table 5.3). For this analysis, two different cohorts (the numerically most abundant cohorts in each population) were compared in each of three populations.

Statistical analysis

Haplotype diversity, analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992), pairwise *F*-statistics, and exact tests of population differentiation were calculated using ARLEQUIN 2.001 (Schneider *et al.*, 2000). In order to address the hypothesis of no spatial or temporal variation in the haplotype or microsatellite allele frequencies, *F*-statistic analogues (Φ_{ST}) were estimated using AMOVA. Values of Φ_{ST} for microsatellite loci were calculated by averaging over the 4 loci. Statistical significance of Φ_{ST} estimates were calculated by generating 10,100 datasets and determining the proportion of occurrences of values greater than or equal to the observed value. The hypothesis of equal haplotype and allele frequencies among cohorts and populations was assessed using exact tests for population differentiation (Raymond and Rousset, 1995a). Inbreeding coefficients (F_{IS}) for the microsatellite data were calculated using FSTAT (Goudet, 2001).

In order to determine whether large samples of single cohorts collected as early life history stages have different allele and genotype frequencies relative to single cohorts collected as adults, microsatellite allele frequencies from both larval samples and newly settled recruits were compared to the adult population samples reported in Chapter Three (Addison and Hart, 2004).

Results

Age structure

Paired dark and light annual rings were easy to score and in all cases the outermost ring was light. This pattern was consistent with the expectation that sea urchins collected in the summer months were growing rapidly in response to seasonal increases in food resources and ocean temperatures. Test diameter was a poor predictor of age (Figure 5.1), which indicated that overall growth was not uniform within or among the five locations. With the exception of the Bear Cove sample, populations of sea urchins collected from the five sites demonstrated considerable age structure (Figure 5.1). Sea urchins ranging in age from 2 to 24 years old correspond to a range of settlement events dating back to 1975. Samples collected at Bear Cove were consistent with population recovery via planktonic recruitment following the 1995 disease epidemic. Only two age classes were present at that site, and they corresponded to settlement in 1999 and 2000.

In contrast, age analysis indicated that samples from East Jeddore had a history of settlement prior to the disease epidemic, which suggests that these sea urchins had re-populated the site from a deep-water population that included a broad range of older year classes (like the other population samples) that were unaffected by the 1995 disease

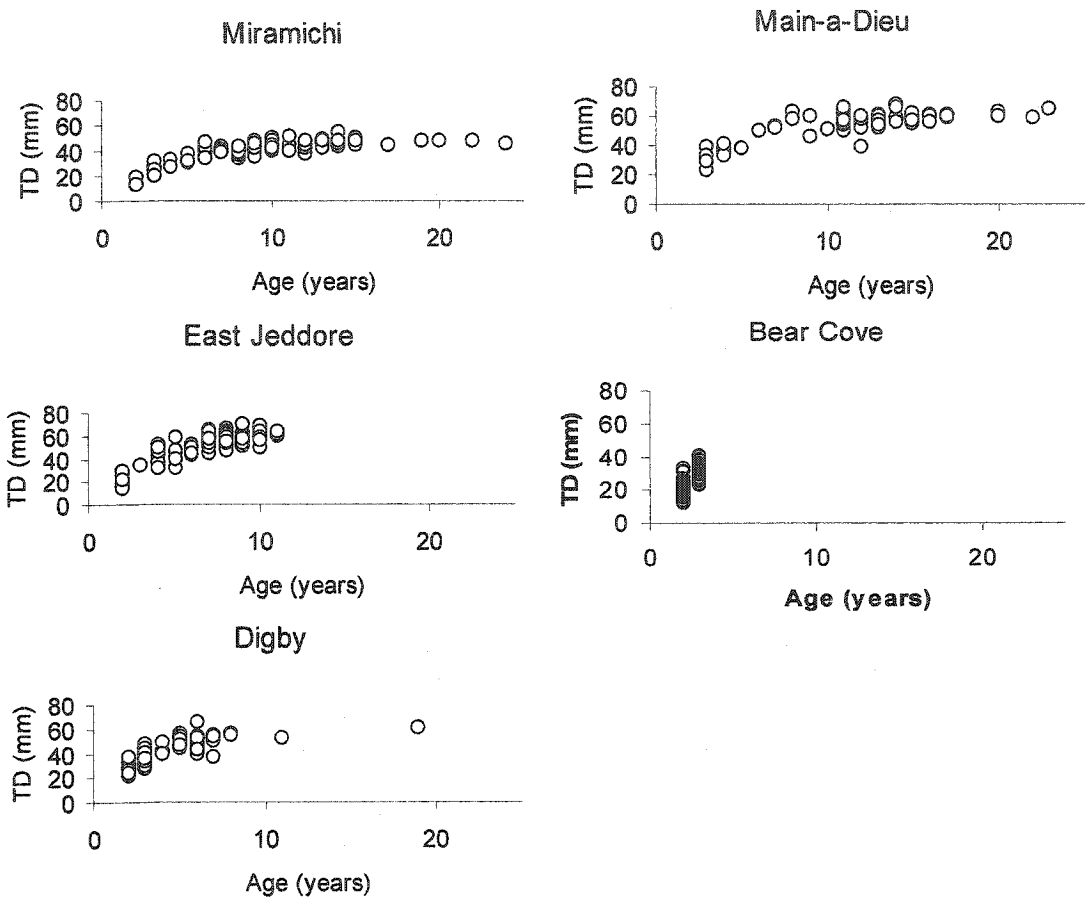

Figure 5.1 The relationship between the test diameter (mm) and the age of individual sea urchins collected from five different populations throughout Atlantic Canada. Axes are drawn to the same scale.

outbreak (A. Baker, personal communication). Direct studies of settlement and recruitment patterns of sea urchins at single sites in the northwest Atlantic suggest that recruitment is highly episodic and major settlement events occur infrequently among years (Harris and Chester, 1996; Balch and Scheibling, 2000; Lambert and Harris, 2000). Broad distribution of many age classes in most populations in this study (Figure 5.1) suggests that spatial mixing of cohorts after recruitment might make a significant contribution to age structure (and perhaps to population genetic patterns) in these older populations, even those recently devastated by disease outbreaks.

Microsatellite genetic diversity

Levels of allelic richness observed in the genotype data from both larvae and new recruits were similar to those reported for multi-age populations in Chapter Three (Addison and Hart, 2004; Appendix 2). Average inbreeding coefficients (F_{IS}) for single cohorts ranged from 0.0110 to 0.2540 (Table 5.1), indicating that heterozygote deficits were common within cohorts. At the population level, larger heterozygote deficits were weakly correlated with increasing number of age classes sampled in the population (R^2 = 0.638; Figure 5.2), but not with increasing age of the sample (R^2 = 0.008). Overall, smaller inbreeding coefficients were detected in the samples of planktonic larvae (F_{IS} = 0.0330) and newly settled recruits (F_{IS} = 0.0700) than were detected for multi-aged populations (F_{IS} = 0.1320-0.2000; Table 5.1, Figure 5.2), and all northwest Atlantic samples combined (F_{IS} = 0.1436; Table 3.2). Only one cohort (the oldest animals collected at Main-a-Dieu, F_{IS} = 0.0110) showed an average inbreeding coefficient as low as those observed for the two samples of young-of-the-year animals collected as larvae or juveniles, and this average mainly reflected a large heterozygote

Table 5.1 Inbreeding coefficients (F_{IS}) calculated separately for each microsatellite locus, combined across loci, per population (Pop.), and overall for different cohorts of sea urchins (location and settlement year) from seven populations. N is the sample size, and values in bold are significantly different from zero based on 1520 permutations of the data.

		*F*IS						
Population/cohort)	N	Sd156	Sd121	Sd76	Sd63	All	Pop.	Total
Miramichi Bay '87	11	0.2000	-0.0950	0.2470	**0.2780**	**0.1550**		
Miramichi Bay '89	11	0.1880	0.0200	0.1880	**0.4380**	**0.2010**		
Miramichi Bay '90	9	**0.4960**	0.1380	**0.2940**	0.0820	**0.2540**	**0.2000**	
Main-a-Dieu '84	9	0.2440	0.1820	0.0940	-0.1030	0.0110		
Main-a-Dieu '86	10	**-0.0520**	**0.2320**	**0.3410**	-0.0190	**0.1290**		
Main-a-Dieu '87	9	**0.4840**	0.1580	**0.3390**	0.0260	**0.2540**		
Main-a-Dieu '88	16	**0.2840**	-0.1030	0.0760	0.1270	**0.0930**	**0.1350**	
Jeddore '89	9	0.0260	-0.0750	0.1180	**0.3700**	0.1090		
Jeddore '90	9	**0.3750**	0.1580	0.0090	0.1320	**0.1730**		
Jeddore '91	26	**0.4590**	-0.0310	0.0240	**0.3600**	**0.2010**		
Jeddore '92	25	**0.1590**	-0.0600	0.1170	**0.3030**	**0.1270**	**0.1570**	
Halifax '02	112	0.0280	-0.0280	0.1050	0.0300	**0.0330**	**0.0330**	
Bear Cove "00	37	0.0900	0.0040	**0.4970**	0.0870	**0.1680**		
Bear Cove "99	47	**0.2020**	-0.1340	**0.2330**	0.1080	**0.1020**	**0.1320**	
Digby '93	16	0.2010	0.0990	**0.3510**	**0.3080**	**0.2370**		
Digby '94	10	0.1820	0.2850	**0.3330**	0.1250	**0.2340**		
Digby '96	32	**0.1820**	0.0770	**0.2330**	0.0530	**0.1370**		
Digby '97	16	**0.2720**	0.0280	0.1710	**0.2610**	**0.1810**	**0.1790**	
Isle of Shoals '94	144	0.0490	-0.0340	**0.1210**	**0.1510**	**0.0700**	**0.0700**	**0.1120**

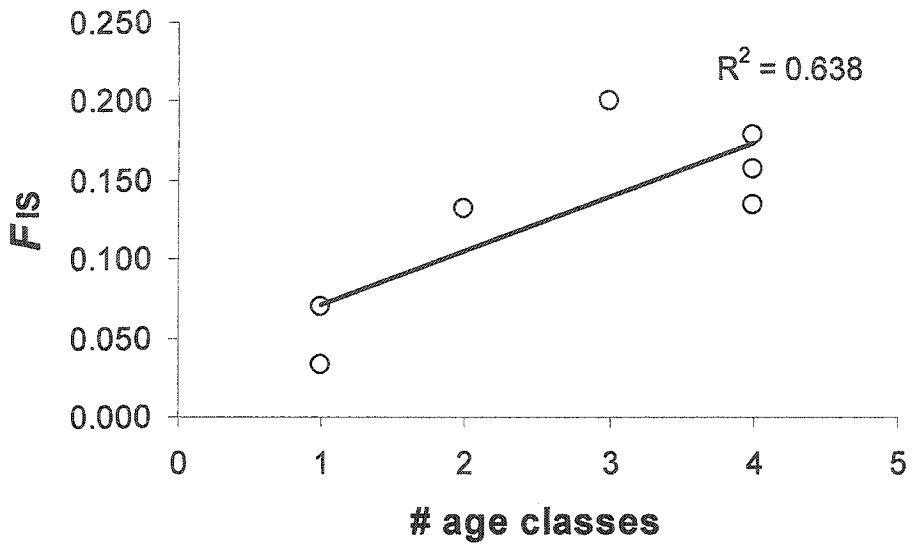

Figure 5.2 The relationship between the population level inbreeding coefficient (F_{IS}) and the pooled number of age classes used for each calculation.

excess at one locus (*Sd*63) with heterozygote deficits at the other three loci.

Samples of sea urchins of different ages exhibited small but significant levels of temporal and spatial genetic heterogeneity. A combined analysis of all 15 year classes (1984-2002) revealed evidence of significant temporal variation in allele frequencies among sea urchins settling in different years ($\Phi_{ST} = 0.0056$, $P = 0.0059$). Pairwise analysis of cohorts (Table 5.2) identified numerous scattered differences based on permutation tests. However, after Bonferoni correction for multiple tests, AMOVA (but not exact tests) indicated only four of these comparisons were statistically significant: all involved contrasts between the sample of larvae from Halifax Harbour (2002) compared to juveniles from Isle of Shoals (1994) or adult cohorts from Miramichi Bay and East Jeddore (1990-1992). Temporal heterogeneity among cohorts settling at the same site was absent among all populations except Main-a-Dieu. Significant genetic differentiation was detected using AMOVA for cohorts settling at this location in 1984 and 1986 ($\Phi_{CT} = 0.0259$, $P = 0.0178$), but exact tests produced non-significant P values.

Samples of sea urchins settling at different sites in the same year did not provide evidence for spatial heterogeneity within year classes (AMOVA $P > 0.1857$; Pairwise Φ_{CT} in Table 5.2). However, there were only four possible comparisons in this analysis (see Table 5.1), and sample sizes were likely too small to provide a reasonable test of spatial structure within age classes. Pooling temporal samples collected at the same site provided evidence for small but significant population genetic structure among the sites included in this analysis ($\Phi_{ST} = 0.0035$, $P < 0.0000$). The addition of these samples to the more extensive analysis of the northwest Atlantic (Chapter Three) resulted in a small but highly significant amount of total genetic structure ($\Phi_{ST} = 0.0028$, $P < 0.0000$). After a correction for multiple tests, pairwise comparisons showed that the sample of larvae and

Table 5.2 Population and age class pairwise Φ_{ST} values calculated in Arlequin 2.001 (lower diagonal) using the four locus microsatellite DNA genotypes from cohorts with sample sizes greater than or equal to nine. P-values (upper diagonal) in bold represent significant differences after 10,100 permutations of the data and values marked by an asterisk (*) were significant after Bonferroni correction for multiple tests. Population abbreviations (and year of settlement) are as follows: Miramichi Bay (MBY), Main-a-Dieu (MAD), East Jeddore (JED), Halifax Harbour (HFX), Bear Cove (BCV), Digby (DBY), and the Isle of Shoals (ISH).

Table 5.2

	MBY '87	MBY '89	MBY '90	MAD '84	MAD '86	MAD '87	MAD '88	JED '89	JED '90	JED '91	JED '92	HFX '02	BCV "00	BCV "99	DBY '93	DBY '94	DBY '96	DBY '97	ISH '94
MBY '87		0.9728	0.3728	0.4696	0.9181	0.8053	0.4763	0.6687	0.6731	0.4354	0.7351	0.0798	0.7512	0.7091	0.7632	0.5895	0.9132	0.8155	0.9635
MBY '89	-0.0176		0.1617	0.3848	0.4594	0.3576	0.5553	0.1158	0.3287	0.3529	0.9544	**0.0023**	0.1061	0.1854	0.2313	0.4439	0.6868	0.1076	0.4962
MBY '90	0.0076	0.0133		**0.0164**	0.1556	0.4436	0.0380	0.1009	0.3433	0.0190	0.6459	***0.0003**	**0.0056**	**0.0015**	0.1196	0.1079	0.1658	**0.0275**	**0.0444**
MAD '84	-0.0102	-0.0086	0.0334		**0.0178**	0.5813	0.2822	**0.0175**	0.2292	0.2480	0.0632	**0.0442**	0.3825	0.1208	0.6114	0.3143	0.3421	**0.0439**	0.2538
MAD '86	-0.0126	-0.0017	0.0113	0.0259		0.8611	0.4433	0.6863	0.6374	0.1906	0.6398	**0.0114**	0.2971	0.6360	0.2161	0.5143	0.8670	0.9567	0.1883
MAD '87	-0.0017	0.0049	0.0070	0.0060	-0.0145		0.6012	0.4541	0.2097	0.1722	0.3550	0.1158	0.5129	0.6462	0.8219	0.8722	0.4187	0.7608	0.5442
MAD '88	-0.0011	-0.0050	0.0175	-0.0064	-0.0048	-0.0147		0.5149	0.3281	0.7731	0.5132	0.1860	0.4310	0.9026	0.0953	0.7863	0.7202	0.2319	0.8178
JED '89	-0.0058	0.0122	0.0050	0.0240	-0.0143	-0.0086	-0.0059		0.4471	0.6860	0.5722	0.0725	0.5067	0.8819	0.1968	0.0635	0.6953	0.7453	0.6173
JED '90	-0.0098	0.0140	0.0110	0.0110	0.0042	0.0299	0.0119	0.0055		0.7076	0.4175	**0.0009**	0.2140	0.5392	**0.0564**	0.6746	0.4178	0.3287	0.5421
JED '91	-0.0028	-0.0018	0.0247	0.0004	0.0053	0.0157	-0.0022	-0.0087	-0.0003		0.7023	***0.0003**	**0.0047**	0.0854	0.0924	0.1719	0.2111	0.1485	**0.0243**
JED '92	-0.0016	-0.0101	-0.0078	0.0141	-0.0051	0.0009	-0.0026	-0.0074	0.0104	-0.0033		***0.0003**	**0.0102**	**0.0260**	0.0675	0.2760	0.8608	0.0997	**0.0129**
HFX '02	0.0020	0.0061	0.0324	-0.0009	0.0114	0.0090	0.0002	0.0061	0.0219	0.0120	0.0149		**0.0012**	**0.0287**	**0.0006**	**0.0102**	**0.0097**	**0.0444**	***0.0003**
BCV "00	-0.0087	0.0061	0.0220	-0.0042	0.0011	-0.0007	-0.0016	-0.0021	0.0047	0.0167	0.0123	0.0037		0.6050	0.2556	0.3263	0.3132	0.1731	0.1500
BCV "99	-0.0003	0.0031	0.0285	0.0102	-0.0050	-0.0025	-0.0056	-0.0069	0.0099	0.0055	0.0063	0.0017	0.0011		0.1877	0.5009	0.5178	0.6626	0.7781
DBY '93	-0.0127	-0.0046	0.0096	-0.0030	-0.0056	-0.0270	-0.0021	-0.0020	0.0200	0.0074	0.0054	0.0090	0.0031	-0.0014		0.1579	0.2076	0.5795	0.2263
DBY '94	0.0002	0.0008	0.0231	0.0082	0.0006	-0.0083	-0.0075	0.0158	0.0009	0.0102	0.0085	0.0108	0.0021	-0.0031	0.0036		0.6386	0.2094	0.4980
DBY '96	-0.0130	-0.0067	0.0076	0.0020	-0.0110	0.0022	-0.0048	-0.0089	0.0040	0.0030	-0.0048	0.0002	0.0005	-0.0033	0.0004	-0.0018		0.7950	0.6234
DBY '97	-0.0093	0.0147	0.0193	0.0291	-0.0176	-0.0077	0.0035	-0.0163	0.0124	0.0121	0.0095	0.0086	0.0067	-0.0012	-0.0043	0.0118	-0.0067		0.1629
ISH '94	-0.0059	0.0020	0.0165	0.0030	0.0066	0.0080	-0.0007	0.0009	0.0067	0.0097	0.0082	0.0038	0.0013	-0.0001	0.0034	0.0036	-0.0029	0.0073	

the sample of recruits were significantly different only from the multi-age adult population sample collected from East Jeddore (Φ_{ST} = 0.0096 and 0.0065, respectively). However, these differences were not statistically significant by exact tests.

Mitochondrial Genetic Diversity

I found 17 COI haplotypes that were broadly distributed among populations and year classes (Table 5.3). Year classes of sea urchins exhibited strong evidence of temporal genetic heterogeneity in haplotype frequencies (Φ_{ST} = 0.1176, P = 0.0002), but pooling the cohorts collected at the same location revealed no evidence of spatial genetic subdivision (Φ_{ST} = 0.0206, P = 0.1053). Both AMOVA and exact tests indicated significant pairwise differences among the cohort that settled in Miramichi Bay in 1989 and the two cohorts from Bear Cove (Table 5.4). Of the three comparisons between cohorts from a single sampling location, the comparison of cohorts that settled at Bear Cove was significant after Bonferoni correction and by the exact test (Φ_{ST} =0.1485, P = 0.0008, exact test P = 0.0190).

Table 5.3 Population (cohort), sample size, COI mtDNA haplotype number, and haplotype diversity (± SE) for the cohorts included in the age structure analysis. Haplotype numbers are the same as those reported in Chapter Four.

Haplotype	Miramichi 1987 (n=10)	Miramichi 1989 (n=9)	Jeddore 1991 (n=18)	Jeddore 1992 (n=24)	Bear Cove 1999 (n=25)	Bear Cove 2000 (n=22)	All NW Atlantic (n=132)
1	1		1	3		5	13
13				1			1
14			1	1			2
15			1				1
16			1		1		2
17				1	2	2	5
18	5	4	8	13	17	9	67
19	1	1	3	2	2	1	12
20	2		3	2	2		11
21				1			1
23		4					8
24						2	3
25	1						1
26						1	1
27						1	1
28						1	1
29					1		1
Haplotype Diversity	0.7556 (0.1295)	0.6667 (0.1048)	0.7778 (0.0835)	0.6993 (0.0966)	0.5367 (0.1152)	0.7922 (0.0687)	0.7163 (0.0390)

Table 5.4 Population and age class pairwise Φ_{ST} values calculated in Arlequin 2.0001 (lower diagonal) using the COI haplotyes frequencies. P-values (upper diagonal) in bold represent significant differences after 10,100 permutations of the data, and Φ_{ST} values in italics were significantly different after exacts for significant differences. Values marked by an asterisk (*) were significant after Bonferroni correction for multiple tests.

	Miramichi '87	Miramachi '89	Jeddore '91	Jeddore '92	Bear Cove '99	Bear Cove '00
Miramichi '87		**0.0371**	0.9495	0.8131	**0.0468**	0.1788
Miramichi '89	0.2982		**0.0187**	**0.0074**	**0.0019**	**0.0704**
Jeddore '91	-0.0548	0.2603		0.6662	**0.0170**	0.1597
Jeddore '92	-0.0390	0.3040	-0.0191		0.1871	**0.0479**
Bear Cove '99	0.1080	*0.4854*	0.1025	0.0145		***0.0008**
Bear Cove '00	0.0359	*0.1008*	0.0375	0.0594	**0.1485*	

Discussion

Many marine species are characterized by the high dispersal life history syndrome. Owing to the prolonged planktonic lives of their gametes and offspring, these species are susceptible to a large variance in individual reproductive success. If the unpredictability of the marine environment results in a small and random fraction of the adult population contributing to each breeding event, then reproductive success can be viewed as a sweepstakes (Hedgecock, 1994). This reduction in the effective number of breeders is expected to decrease levels of genetic diversity within cohorts of recruits (Hedgecock, 1994), and the random sampling of a subset of adult breeders is expected to result in genetic differentiation among cohorts spawned in different breeding events separated from each other in time or space (Li and Hedgecock, 1998).

My genetic analysis of age structure in sea urchins does not strongly support the first prediction of the sweepstakes hypothesis: there was little evidence for a reduction in genetic diversity within age classes relative to multi-age populations. However, significant genetic differentiation in haplotype frequencies among cohorts of adult sea urchins provides strong evidence that females experience the large variance in reproductive success proposed by the sweepstakes hypothesis. The large amount of among cohort variability in the absence of spatial structure is striking, and although local scale differentiation could be generated by differential patterns of mortality either before or after recruitment, this pattern is also consistent with the predictions of large variance in reproductive success.

The subtle patterns of genetic variation in the microsatellite data were also consistent with the sweepstakes hypothesis. First, allele frequency differences at the four microsatellite loci revealed significant genetic variation among the different age classes

of sea urchins, and the magnitude of temporal variation (Φ_{ST} = 0.0056) was similar to the magnitude of spatial variation (Φ_{ST} = 0.0035). This pattern is predicted if the processes that determine which subsets of adults contribute to each spawning event are random with respect to genotype. Second, if heterozygote deficits are generated by the accumulation of genetically distinct cohorts of new recruits, then this unrecognized population structure will lead to a temporal Wahlund effect. This prediction is weakly supported by the positive correlation between increasing age structure and larger heterozygote deficits in the microsatellite data (Figure 5.3). However, much of this correlation is driven by the large difference between heterozygote deficits in two samples of early life history stages (low F_{IS}) and 17 samples of adult cohorts (high F_{IS}) (Table 5.1). One interpretation of this difference is that much of the reduced heterozygosity observed in cohorts as adults (Table 5.1) results from spatial mixing of genetically differentiated pools of offspring after recruitment of larvae into benthic adult populations. Such within-population structure could be produced, for example, by extensive migration of benthic adults away from the location to which they recruited as new juveniles.

One potentially significant source of genetic variation among cohorts could arise as soon as cohorts are formed: through variation in fertilization success of spawning adults. Because sperm limitation may reduce overall fertilization success in some marine invertebrates (Yund, 2000; Marshall, 2002), both male and female adults may vary widely in reproductive success measured by the size of the zygote pool (i.e., overall reduction in the effective population size). If male success varies more than female success, then biparental genetic markers (microsatellites) might be expected to show greater among-cohort variation than maternally inherited markers (mtDNA). I found more consistent and significant temporal variation at the mtDNA locus. Alternatively, if

male and female reproductive variation is similar, the mtDNA markers might be expected to show greater temporal variability due to the much smaller effective size of this marker pool (inherited through half of adults as a haploid locus; Wilmer *et al.,* 1999). In addition, the sample sizes used to characterize patterns in microsatellite allele variability were relatively small. A simulation study by Ruzzante (1998) suggests samples of 50 or more individuals per population in order to reduce the bias associated with the estimates of *F*-statistics. The sample sizes included in my analysis ranged from 2 to 144 per cohort (age class/population). In contrast to the strong support provided by the mtDNA analysis, the patterns of genetic differentiation I report here for the microsatellite data do not provide unequivocal support for the sweepstakes hypothesis. However, where significant, the patterns are consistent with the sweepstakes predictions. Larger cohort sample sizes are required to further disentangle the potential effects of differential male and female variance in reproductive success.

I found significant genetic structure among different cohorts of *S. droebachiensis* using the same maternally inherited marker (COI) that Flowers *et al.* (2002) used to document genetic homogeneity among cohorts of *S. purpuratus*. While this contrasting result could be due to differences in the community ecology of the two species on the different coasts, it could also be due to differences in overall haplotype diversity: 0.919 – 0.949 for age classes of *S. purpuratus* in the northeast Pacific (Flowers *et al.,* 2002) versus 0.537-0.792 for *S. droebachiensis* in the northwest Atlantic (Table 5.3). My phylogeographic study (Chapter Four), which is based on parsimony and maximum likelihood analyses of the same COI sequences, shows that most northwest Atlantic *S. droebachiensis* COI haplotypes are recently derived from a single haplotype shared in common with older and genetically more diverse north Pacific populations. As a result,

the biogeographic history of colonization and range expansion in Atlantic *S. droebachiensis* may have caused reduced genetic diversity in those populations in comparison to Pacific *S. purpuratus*. Fewer haplotypes in Atlantic populations probably improved the chances of detecting random drift among offspring from different spawning events.

Previous empirical studies investigating the sweepstakes hypothesis have focused on genetic subdivision among cohorts (Li and Hedgecock, 1998; Ruzzante *et al.*, 1996; David *et al.*, 1997; Moberg and Burton, 2000), kinship among larvae (Herbinger *et al.*, 1997; Boudry *et al.*, 2002), and the measurement of genetic drift in natural populations (Hedgecock, 1994). It has been difficult to measure the significance of variance in reproductive success as a general mechanism that lowers N_e, in part because such an effect can be confounded with the effects of variable strength and direction of gene flow among genetically differentiated populations of reproductive adults that cause temporal variation among cohorts recruiting to the same location (e.g., Kordos and Burton, 1993; Benzie and Williams, 1997). However, given the low levels of spatial genetic structure in the northwest Atlantic populations of sea urchins, it seems unlikely that the genetic differences among cohorts of new recruits are produced solely by the co-recruitment of offspring from genetically differentiated spawning adult populations.

If high variance in reproductive success is common in marine invertebrates with a high dispersal life history syndrome, then why are the observed levels of genetic drift among populations (F_{ST}) so small? One possibility is that the influence of sweepstakes reproduction is obscured by patterns of gene flow, particularly in species with extremely long lived planktonic larvae, and that the net effect of sweepstakes variation on natural populations could be relatively small. Without direct measures of individual reproductive

success and its variance, the evidence for sweepstakes reproduction is limited to indirect insights from patterns of genetic structure among age classes and levels of heterozygote deficiency in multi-aged adult populations. Both of these values are influenced by stochastic events affecting the dispersal ability of offspring (gene flow) and the magnitude of individual variance in reproductive success.

These interacting influences are summarized schematically in Figure 5.3. Studies of sweepstakes reproduction have mostly been motivated by empirical observations of heterozygote deficits, high F_{IS} values, and deviations from Hardy-Weinberg equilibrium genotype expectations (the starting box in the upper left of Figure 5.3). Such patterns could be caused either by spatial structure within the functional sampling units that are identified as populations (the classic Wahlund effect) or by genetic variation among age classes (the temporal Wahlund effect). Identification of temporal genetic variation by direct examination of cohorts is necessary but not sufficient to test the sweepstakes reproduction hypothesis because temporal variation in the source of recruits (from genetically differentiated adult populations) can also produce such age structure (the diagonal arrow in Figure 5.3). If this latter effect cannot be ruled out, then direct observations of variation in reproductive success are needed to estimate the direct influence of sweepstakes reproduction on age structure and heterozygote deficits in age-structured populations.

A second explanation for the low levels of observed population structure in some marine invertebrates, particularly sea urchins, could be due to the longevity of these organisms. Both Murphy (1968) and Ebert (1975) suggest that life history and demographic characteristics of sea urchins have evolved in response to the large variance

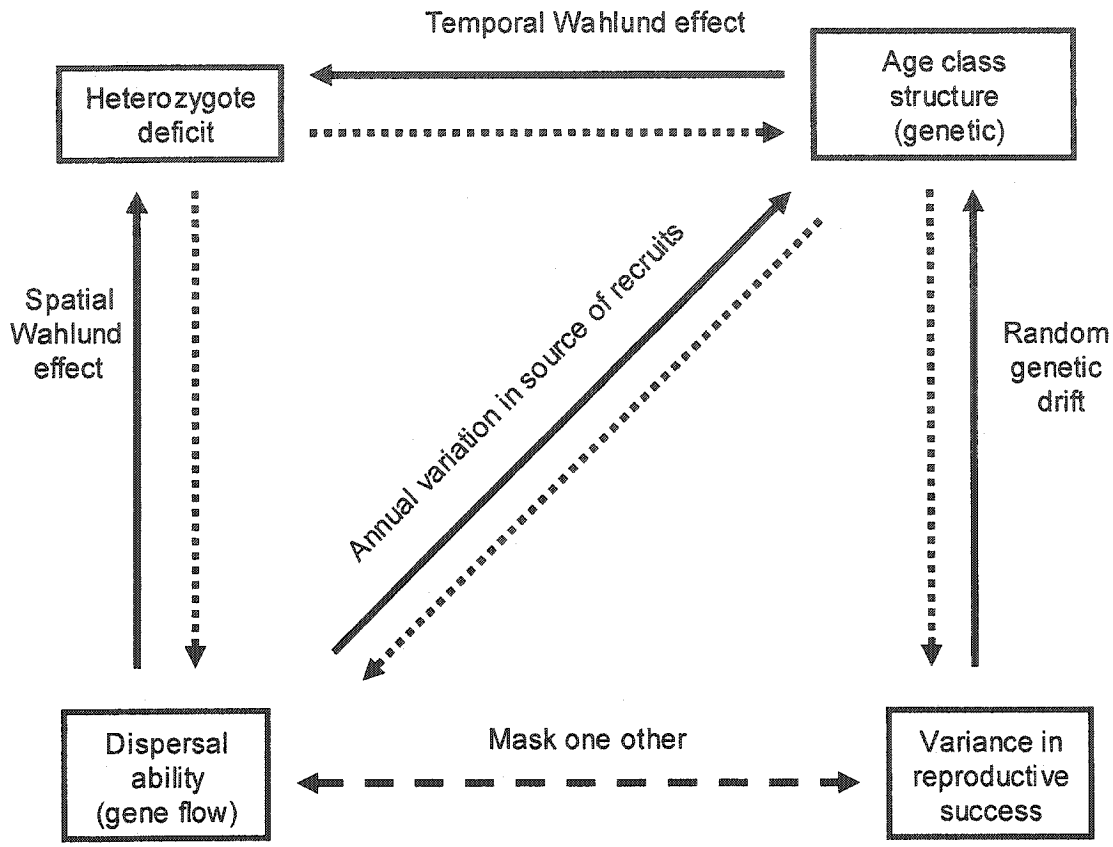

Figure 5.3 Graphical representation of relationships between two measured population genetic variables and the random population level processes that influence them. Solid arrows represent causative effects and dotted arrows represent patterns in the data that can support the hypotheses of the population level processes. The dashed line indicates a confounding relationship among the two hypotheses.

in reproductive success. High rates of annual reproductive failure caused by low fertilization rates or high mortality of pre-reproductive stages (e.g., larvae and juveniles) will favour long adult life and the distribution of reproductive efforts over many years (i.e., bet hedging). As observed in this study and many others, increased longevity in sea urchins results in the generation of extensive age class structure. If variance in reproductive success causes annual cohorts of sea urchins to be genetically differentiated, then the accumulation of many age classes at a given location may act to buffer this differentiation and prevent the loss of alleles due to random sampling during breeding events in which the effective number of spawning adults is low. Ebert (1975) suggested that the evolution of longevity in sea urchins acts to decrease the individual lifetime variance in reproductive success. Thus, diversity of neutral genetic markers sampled in age-structured populations may not reflect the annual or episodic reduction in N_e associated with sweepstakes reproduction if the overall lifetime reduction in N_e is limited by bet hedging. Therefore, high levels of gene flow and large lifetime N_es in long-lived age structured populations may effectively compensate for annual variance in reproductive success and result in high within-population genetic diversity and low levels of genetic drift among populations. Species with short adult life spans or semelparous reproduction might be expected to more often show the influence of sweepstakes reproduction on population heterozygosity and genetic variation among cohorts.

CHAPTER SIX

BROADCAST SPAWNING OF SPERM IS CORRELATED WITH HIGH INBREEDING COEFFICIENTS IN MARINE INVERTEBRATES

Introduction

Thousands of marine invertebrate species have large census population sizes, high fecundity, and planktonic larvae capable of broad dispersal in ocean currents, while closely related species in the same higher taxa have evolved encapsulated or viviparous development without larval dispersal (Strathmann, 1985; Pechenik, 1999). This dramatic life history variation has attracted the attention both of population geneticists interested in gene flow and of marine ecologists interested in larval phenotypes. This reproductive variation includes a number of independent, covarying characteristics of spawning, development, and larval morphology. However, population genetic studies of marine invertebrate life history variation have focused mainly on measuring among-population differentiation (Wright's F_{ST}) relative to one particular reproductive trait: the potential dispersal of individuals between populations during the planktonic larval phase (reviewed by Knowlton and Jackson, 1993; Palumbi, 1995; Bossart and Prowell, 1998; Benzie, 1999; Bohonak, 1999; Grosberg and Cunningham, 2001; Hellberg *et al.,* 2002).

Knowlton and Jackson (1993) emphasized a subtly different interpretation of Wright's F statistics in the context of marine invertebrate reproductive variation: as measures of the random component of inbreeding and loss of heterozygosity due to genetic drift among differentiated populations (F_{ST}), and the non-random component of inbreeding (F_{IS}) due to non-random matings within populations or due to other processes

that contribute to genetic structure on a spatial scale smaller than the sampling units identified as populations. Knowlton and Jackson (1993) reviewed life history traits of marine invertebrates that vary among species or higher taxa and could influence the random component of inbreeding (F_{ST}). In general, the predicted effect of variance in larval dispersal on the random component of inbreeding and loss of heterozygosity has been borne out in empirical studies. The broad exchange of larvae and genes between far-flung parts of the ocean (Lessios *et al.*, 1998) should reduce the random component of inbreeding (F_{ST}) by homogenizing genetic differences that arise due to mutation and drift. These effects have been observed as a general negative correlation between potential for planktonic larval dispersal and F_{ST} values (e.g., Bohonak, 1999), but significant departures from this expectation have been frequently observed (e.g., Johnson and Black, 1982).

Marine population geneticists have paid less attention to among-species variation in the non-random component of inbreeding (F_{IS} or its analogs, sometimes referred to simply as the inbreeding coefficient) that is observed within population samples that are assumed to experience random mating among all members of each sampled population and satisfy the other assumptions of population genetic equilibrium conditions (Knowlton and Jackson, 1993). High F_{IS} values are frequently explained in terms of laboratory artifacts or unobservable null alleles (e.g., Lundy *et al.*, 1999, 2000) that lead to low levels of observed heterozygosity within populations relative to equilibrium predictions of heterozygote frequencies. Planned comparisons of inbreeding coefficients are sometimes restricted to studies of species without extensive larval dispersal (e.g., Cohen, 1996) in which mating might be locally non-random on a scale smaller than that of typical larval

dispersal and smaller than the typical spatial scale of population genetic sampling (Whitaker, 2004).

Marine invertebrate population genetics studies that were focused on among-population differentiation often report (sometimes as a parenthetical aside) a low frequency of heterozygotes relative to Hardy-Weinberg equilibrium expectations and a high F_{IS} value significantly different from zero; many of these reports come from studies of species with broadly dispersing larvae (e.g., Chapter Three; Addison and Hart, 2004; see Hellberg *et al.,* 2002) in which the random component of inbreeding is small and F_{ST} values on relatively large spatial scales are not significantly different from zero. There has been no general review of these F_{IS} values and no attempt to offer a mechanistic explanation for them except as laboratory artifacts or products of selection (both of these processes are known to contribute to heterozygote deficiencies in some particularly well-studied systems; e.g., Zouros and Foltz, 1984; Foltz and Hu, 1996; Raymond *et al.,* 1997). For example, large heterozygote deficits and high inbreeding coefficients are often measured in studies of bivalves (clams, oysters and mussels) and anthozoans (anemones and corals), and these observations are consistently interpreted as being a result of selection or null alleles.

One candidate explanation that has received increasing attention is the effect of fertilization biology on allele and genotype frequencies. Broadcast spawning marine animals frequently experience severe sperm limitation, with low and variable rates of fertilization, in which a few spawners achieve high fertilization rates while many others fail to contribute alleles to the pool of zygotes (Hedgecock, 1994; Levitan, 1996, 2002; Li and Hedgecock, 1998; Yund, 2000). Recent field experiments measuring individual fertilization success in *Strongylocentrotus* sea urchins (D. Levitan, unpublished ms) show

that individual fertilization success can be highly variable within spawning groups, and that males vary much more than females in fertilization success. Population genetic theory predicts that such reductions in effective population size (N_e) will produce cohorts of zygotes with relatively high heterozygosity (Pudovkin *et al.,* 1996; Luikart and Cornuet, 1999).

Hedgecock (1994; Li and Hedgecock, 1998) proposed an additional effect of highly variable fertilization success among individual freespawners: high variation in allele frequencies among cohorts that are produced by different spawning groups. Such temporal variation in allele frequencies among age classes has been invoked to explain heterozygote deficits (and high F_{IS} values) in age-structured populations with free-spawning and broad larval dispersal in which spatial differentiation of populations is absent or transient (e.g., Watts *et al.,* 1990; David *et al.,* 1997; Planes and Lenfant, 2002). Hedgecock's hypothesis invokes non-random mating through variance in individual contributions to the zygote pool, combined with significant genetic variation among cohorts of zygotes, to explain local within-population deficits of heterozygote genotypes (high F_{IS}) with low levels of random inbreeding measurable as genetic drift among populations (low F_{ST}) (e.g., Watts *et al.,* 1990; Edmands *et al.,*1996).

I reviewed recent reports of inbreeding coefficients for marine invertebrates and compared mean inbreeding levels with respect to three specific reproductive traits: presence or absence of a widely dispersing larval stage, mode of male spawning (by free-spawning in the plankton or by copulation or some other form of direct transfer of sperm to eggs), and mode of female spawning (by broadcast spawning or by fertilization of benthic eggs). Some other meta-analyses have focused on correlations between male spawning mode and other reproductive characteristics (e.g., Beck, 1998), but not on the

relationship with population traits such as inbreeding coefficients. I focused on the population genetic characterization of inbreeding as the F_{IS} value, and I ignored the large, parallel field of inbreeding measured as phenotypic variation or inbreeding depression (Charlesworth and Charlesworth, 1987; Mitton, 1993; Raymond *et al.*, 1997; Coltman and Slate, 2003).

I found no correlation between inbreeding coefficients and larval dispersal potential (consistent with the prediction that gene flow and genetic drift should be associated with the random but not with the non-random component of inbreeding). I did find a surprising pattern of relationships between inbreeding coefficients and spawning mode: species with free-spawning males have higher mean inbreeding coefficients than species with copulation or some form of sperm transfer. However, this pattern was not associated with the mode of female spawning: inbreeding coefficients were similar for species in which females spawn eggs into the plankton and those in which eggs are fertilized inside the female or in benthic egg masses.

Literature review and analysis

I surveyed the marine population genetics literature for studies that reported F_{IS} values from sampling designs that covered a large proportion of the species range, included multiple nuclear DNA markers, and had large average sample sizes per geographic population. I concentrated on recent papers published since two influential reviews of allozyme studies (Burton, 1983; Zouros and Foltz, 1984) in the hopes of reviewing contemporary allozyme and microsatellite estimates of inbreeding coefficients. I did not include marine fishes because fish studies frequently focus on commercially harvested species in which F_{IS} values could be related to intensive commercial

exploitation. I concentrated my survey on the journals *Marine Biology*, *Molecular Ecology*, and *Evolution*. Although the survey was not exhaustive, it was conducted in ignorance of any correlation between F_{IS} values and reproductive traits. Most of the studies in the review were scanned for F_{IS} values without specific knowledge of the reproductive biology of the organisms. A smaller number of specific studies on organisms with known reproductive traits were added without prior knowledge of the F_{IS} values that were reported. In each case I used the mean estimate of F_{IS} for all populations averaged across multiple loci.

I analyzed these data using individual studies as data points. I scored each species for five binary reproductive characters: mode of larval dispersal (long-lived planktonic larva versus benthic development or brief planktonic period of a few hours or less), male spawning mode (free-spawning sperm into the plankton versus copulation or some other form of direct transfer of sperm to benthic eggs), female spawning mode (broadcasting eggs into the plankton versus fertilization of eggs inside the female or in benthic egg masses), asexuality (with or without a significant asexual stage in the life cycle), and breeding system (dioecious or with some form of hermaphroditism). I compared F_{IS} values for pairs of character states by two-sample randomization tests with 10,000 permutations using Rundom Projects 1.1 (Jadwiszczak, 2003; see Manly, 1997).

Results

F_{IS} values ranged from -0.536 to 0.995 (Appendix 1). Most studies (99) used allozyme markers; others used microsatellites (8) or nuclear DNA sequences (1). One potential concern about the preponderance of allozyme studies is the potential influence of allozyme laboratory artifacts (including somatic aneuploidy in specific tissues, gene

silencing via imprinting, and null alleles that fail to interact with the substrate during staining) on the patterns described below. All of these artifacts could artificially depress measures of heterozygosity and inflate estimates of inbreeding coefficients in some allozyme studies. Allozymes and microsatellites are susceptible to different types of errors because the polymorphisms are scored from electrophoretic variation in enzyme activity versus variation in PCR product size (Avise, 1994; Foltz and Hu, 1996). This potential concern might be partially satisfied if the two types of markers (and their distinctive methodological limitations) gave similar estimates of inbreeding coefficients. For example, Neff and Gross (2001) found a positive correlation between allozyme and microsatellite heterozygosity in a large review of AC microsatellite repeat loci among 39 vertebrate species.

The mean (variance) F_{IS} value for allozymes was 0.165 (0.044); the mean for microsatellites was 0.212 (0.041). For two species (the tunicate *Botryllus schlosseri* and the abalone *Haliotis rubra*), I found an F_{IS} value based on allozymes and another based on microsatellites (Appendix 1): in *Botryllus* the two markers gave similar results, but the allozyme estimate was slightly higher than the microsatellite estimate; in *Haliotis* the allozyme estimate was much lower.

Other comparisons between allozyme and microsatellite results required contrasts between less closely related species or genera (Appendix 1). Two allozyme estimates from the penaeid shrimp *Penaeus monodon* were lower than a microsatellite estimate in *Litopenaeus setiferus*. A microsatellite estimate for the nudibranch sea slug *Goniodoris nodosa* was intermediate between two allozyme estimates for the distantly related sea slugs *Aplysia californica* and *Siphonaria kurracheensis*. A microsatellite estimate for the sea urchin *Strongylocentrotus droebachiensis* was higher than an allozyme estimate for

another sea urchin (*Evechinus chloroticus*) but in the middle of the range of allozyme estimates from 15 studies of sea stars. Thus, although I found relatively high F_{IS} values in some species groups based on numerous allozyme studies (see below), there is no obvious indication among the studies I reviewed of a strong bias toward higher allozyme estimates of inbreeding coefficients due to allozyme artifacts.

Studies were broadly distributed among marine invertebrate phyla: I found 29 F_{IS} estimates from cnidarians (all from the class Anthozoa: stony corals, soft corals, and sea anemones), 21 echinoderms (sea stars, sea urchins, and their relatives), 2 tunicates (both in *Botryllus schlosseri*), 10 crustaceans (especially crabs and shrimp), 43 molluscs (squids, snails, and bivalves), 2 polychaete annelids, and 1 nemertean worm.

Larval dispersal

Mean (variance) F_{IS} was 0.142 (0.032) among 76 studies of species with planktonic dispersal of larvae during some period of premetamorphic development (Figure 6.1A). Mean F_{IS} was 0.232 (0.068) among 32 studies of animals that lack a planktonic dispersing larva or have limited dispersal of a few hours. This small difference was not statistically significant by the randomization test ($P = 0.115$).

Male spawning

Mean F_{IS} was 0.204 (0.055) for 76 studies of species with free-spawning of sperm (Figure 6.1B). In contrast, mean inbreeding coefficient was 0.081 (0.006) among 32 studies of species with direct sperm transfer or copulation ($P = 0.004$); this difference is significant after a Bonferroni correction for multiple simultaneous randomization tests (Sokal and Rohlf, 1995). The magnitude and direction of this difference is striking, and it

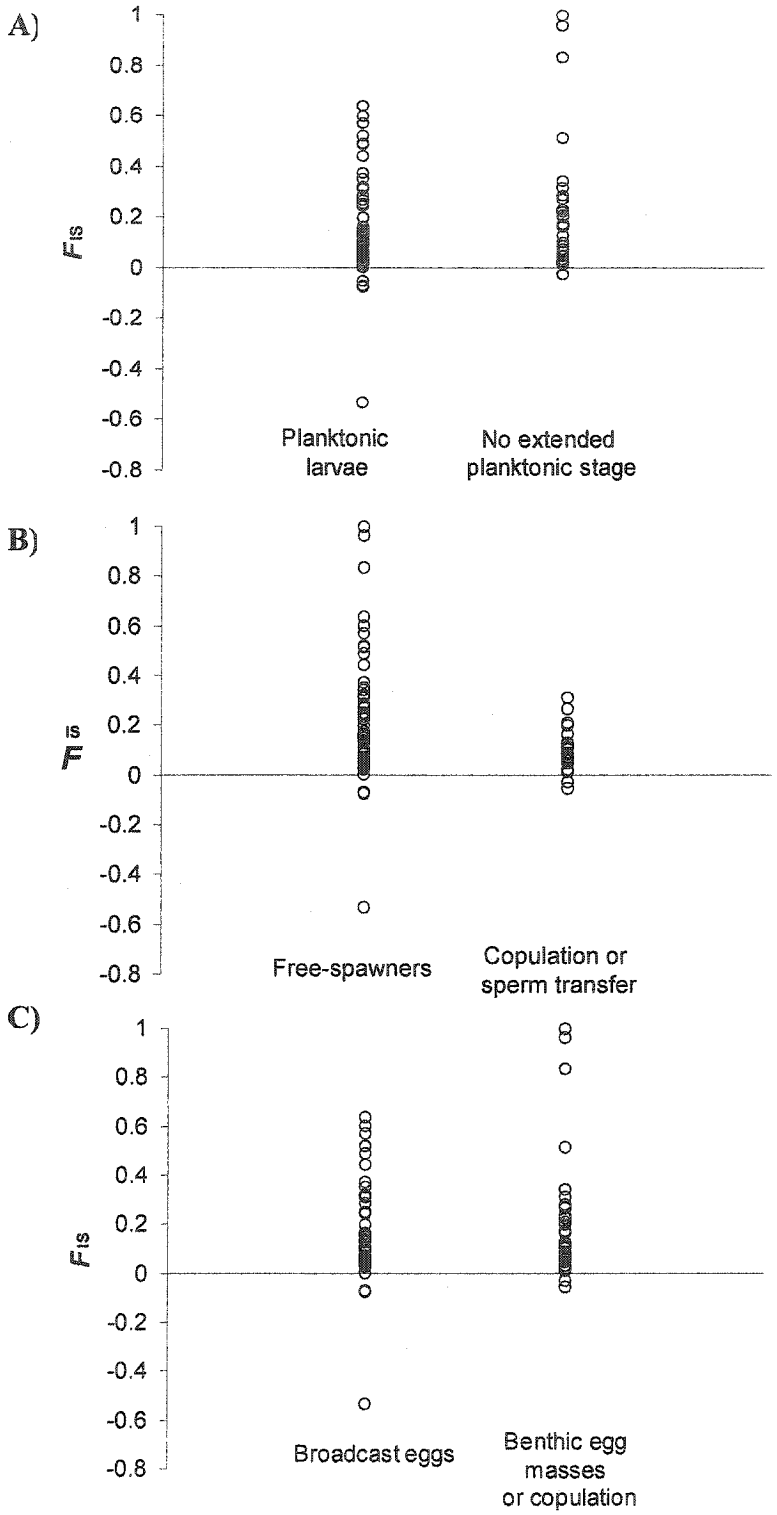

Figure 6.1 Inbreeding coefficients (F_{IS}) of species with **(A)** different larval dispersal abilities, **(B)** different modes of male reproductive ecology, and **(C)** different modes of female reproductive ecology.

is not confounded with either taxonomic affinity or with other reproductive differences (see examples in Appendix 1). Among the 20 highest F_{IS} values (> 0.30) in the survey, 19 came from species with free-spawning males: 12 from anthozoans, three from bivalves, two from gastropods, and one each from polychaetes and tunicates. Species with free-spawning males include some (e.g., echinoderms) with broadcast spawning of eggs by females and others (e.g., some corals) with planktonic sperm but internal fertilization of eggs (a breeding system known as 'spermcasting'; Pemberton *et al.*, 2003b). Free-spawning by males is found in species with and without larval dispersal (both in corals). Species with direct sperm transfer include some (e.g., brooding amphipods) without a planktonic larval stage, and others in which internally fertilized eggs later become postzygotic dispersing larvae (e.g., many gastropods with copulation, early development in egg masses, and later larval development in the plankton). Thus, male spawning mode is only partially correlated with female spawning mode and with larval dispersal ability. Only one combination of these traits was not observed: females that broadcast spawn their eggs are not fertilized by copulation.

Female spawning

My review suggests that this effect of male spawning mode on F_{IS} values is driven by processes influencing the reproductive ecology of males but not of females. The comparison of male spawning modes above could be confounded by variation in female spawning modes. However, I found no significant effect of female spawning mode (Figure 6.1C). The three highest F_{IS} values were found among species without broadcast spawning of eggs, but many relatively high F_{IS} values (0.2-0.6) were found among species that release their eggs into the plankton. Mean F_{IS} was 0.167 (0.037) among 61

studies of females with broadcast spawning of eggs and 0.168 (0.053) among 47 studies of internally fertilized females ($P = 0.980$).

Other factors influencing F_{IS} values

The significantly higher mean inbreeding coefficients for species with broadcast spawning males do not appear to be caused by the sampling design of the various studies (or by other obvious features of the organisms that could be related to within-population genetic variation). One study feature that could lead to high F_{IS} values is sample size: studies with small samples per population are more likely to underestimate the true frequency of heterozygotes and overestimate the non-random component of inbreeding (Ruzzante, 1998). F_{IS} values were weakly negatively correlated with average population sample size ($r = -0.175$; Figure 6.2). Some high F_{IS} values came from studies with small average samples of 20-50 individuals per population (Appendix 1), but many studies with small sample sizes also reported low F_{IS} values, and sample size explained only about 3% of the variation in F_{IS} values among studies.

Other biological factors including asexual reproduction and hermaphroditism could contribute to the over-representation of some genotypes within a population relative to predicted equilibrium genotype frequencies and influence F_{IS} estimates. However, randomization tests suggested non-significant differences between mean F_{IS} values for species with and without significant asexual reproduction ($P = 0.091$; Fig 2.3A) or for species with hermaphrodite and dioecious breeding systems ($P = 0.140$; Figure 2.3B). Unfortunately, my review does not provide a powerful test of either hypothesis. First, only three species (the sea anemone *Epiactis fernaldi* and two species of *Pecten* scallops) are known to be simultaneous hermaphrodites in which selfing is likely and could

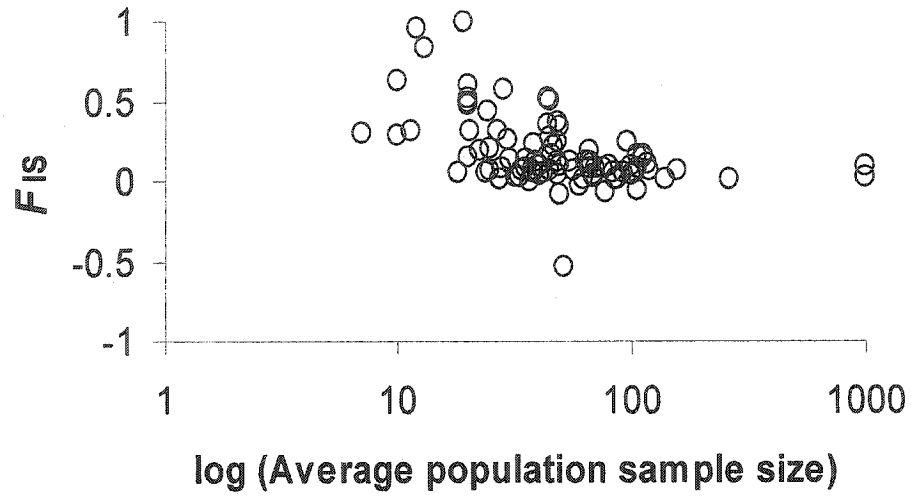

Figure 6.2 The distribution of inbreeding coefficients (F_{IS}) from each study in relation to the average sample size per population

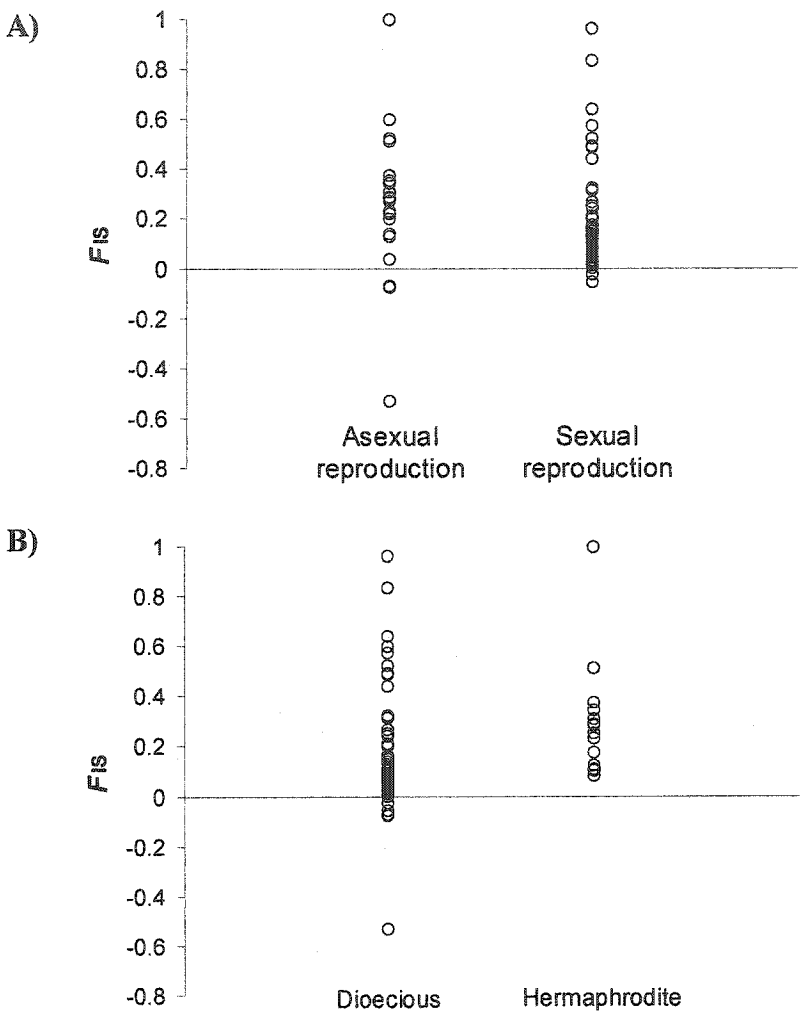

Figure 6.3 Inbreeding coefficients (F_{IS}) of species **(A)** with and without significant asexual reproduction and **(B)** with dioecious or hermaphrodite breeding systems.

influence the evolution of non-equilibrium genotype frequencies relative to allele frequencies. Most other hermaphrodite species in my review are known or likely sequential hermaphrodites in which selfing is poorly studied (e.g., the sea star *Patiriella exigua*). Second, some studies of species with known significant asexual reproduction have attempted to distinguish the effects of asexuality on population genetic parameters, for example by restricting quantitative analysis of allele frequencies to include only unique multilocus genotypes (e.g., Burnett *et al.*, 1995).

Discussion

I found a surprising relationship between inbreeding coefficient and the mode of male spawning: species in which males free-spawn their sperm have higher mean inbreeding coefficients than species with some form of direct sperm transfer or copulation. Similar patterns were not evident with the mode of female spawning: mean F_{IS} values were similar for species that spawned their eggs into the plankton, had internal fertilization, or had fertilization of eggs in a benthic egg mass. I also found no correlation between mean inbreeding coefficients and larval dispersal potential. If a large variance in reproductive success is a common feature of free-spawning marine invertebrates, then my review suggests these patterns are primarily driven by male spawning ecology.

A critical assumption of this analysis is that treating species as data points provides a robust test for the influence of evolved biological traits on the magnitude of the observed inbreeding coefficients. For example, a potential limitation on the interpretation of the highly significant randomization test results is that the higher mean F_{IS} values for free-spawning males are heavily weighted by two taxa (anthozoans and bivalves) in which copulation has not evolved and researchers have reported a large

number of relatively high F_{IS} values. These taxa and others may share similar reproductive traits (and similar inbreeding coefficients) due to their recent shared ancestry and not due to similar adaptive correlations between reproduction and population genetic structure.

In order to account for these and other similar phylogenetic effects, the data were reanalysed using independent contrasts (e.g., Felsenstein 1985; Pagel and Harvey 1988; Harvey and Keymer 1991). In an unpublished collaboration (Addison *et al.*, 2004) we constructed a phylogeny of the species in this review and used the squared-change parsimony method (Maddison 1991) in MacClade to infer ancestral values of F_{IS} (Maddison and Maddison 1999). We used accelerated transformations in MacClade to infer the ancestral states of the three discrete reproductive characters to identify pairs of clades or sister species with different modes of larval dispersal or spawning. Paired *t*-tests of F_{IS} differences for each of these three reproductive characters gave results that were qualitatively the same as the randomization procedures using species as data points in this review (Addison *et al.*, 2004). Evolutionary changes in inbreeding coefficients were significantly associated with evolutionary changes in mode of male spawning, but not with evolutionary changes in mode of larval dispersal or of female spawning. The overall taxonomic or phylogenetic influence was minimal in the analysis of independent contrasts, and this result supports the inferences based on analysis of individual studies as data points in this review.

Larval dispersal and inbreeding

The loss of planktonic larval dispersal should often result in limited gene flow between populations, enhanced genetic drift, and a larger random component of

inbreeding (Knowlton and Jackson, 1993). One potential connection between larval dispersal and non-random inbreeding depends on highly philopatric recruitment. If non-planktonic siblings frequently settle near and mate with each other or their parents, then such local non-random matings between kin could contribute to genetic structure within populations (Grosberg, 1991), and could be manifest as heterozygote deficits and large positive F_{IS} values (e.g., Whitaker, 2004). I found high variation in inbreeding coefficients among species or clades with and without a dispersing planktonic larval stage (Figure 6.1A). Some examples of high inbreeding coefficients, in groups such as some corals with limited larval dispersal between reefs, could have arisen as a consequence of philopatry and consanguineous matings, as suggested by Whitaker (2004), but I found no consistent pattern in F_{IS} values with respect to the presence or absence of a dispersing larval stage.

Planktonic gametes and inbreeding

Why have so many studies of species with planktonic sperm identified relatively high inbreeding coefficients, and why is this effect not also seen in comparisons of species with planktonic versus non-planktonic eggs? These two results appear to reflect sex-biased differences among species in the degree of non-random mating within populations. Such differences are unlikely to be produced by matings among close relatives in species with broadcast spawning of both eggs and sperm because such species also typically have long-range larval dispersal: such larvae tend to diffuse away from their siblings and disperse away from their parents, with limited subsequent opportunities for mating with close kin (Strathmann, 1985; Pechenik, 1999). This sex-biased pattern is also unlikely to be caused by other factors frequently suggested to account for

heterozygote deficits and high F_{IS} values in other reviews. Natural selection against heterozygotes (i.e., outbreeding depression) seems unlikely as a general explanation for this pattern because there is no obvious mechanism that would tend to cause selection against heterozygotes mainly in species with broadcast spawning of sperm. A selection-based explanation of F_{IS} variation among the species I surveyed would have to simultaneously account for (1) similar F_{IS} values between species with and without planktonic eggs or larvae and (2) higher F_{IS} values among free-spawning males relative to copulators, regardless of female spawning mode and larval dispersal abilities. Thus, while some cases of heterozygote deficiencies and high F_{IS} values doubtlessly reflect selection acting on allozyme variation (Burton, 1983; Raymond *et al.*, 1997), selection is probably not a general explanation of this particular pattern.

The generally similar results from allozyme and microsatellite measures of inbreeding coefficients also argue against laboratory artifacts as the major explanation of the patterns I observed: the two types of markers are subject to different potential artifacts that could reduce observed heterozygosities (and lead to artificially high F_{IS} values), but gave similarly broad estimates of F_{IS} values in my review and in others (Neff and Gross, 2001). Also, laboratory artifacts are not expected to produce spurious patterns with respect to one life history trait (mode of male spawning) but not others. The most likely way in which such an artifactual pattern could be produced would be via a large number of biased F_{IS} values observed in some taxonomic groups with free-spawning males in which, for example, allozyme variation can be affected by gene silencing or null alleles. The comparable results using species as data points (in which this artifact could be important) and using pairs of clades that differ in spawning mode (in which this artifact would be less significant) suggest that the results are not strongly biased by, for example,

artifacts associated with allozyme heterozygote frequencies measured in anthozoans and bivalves (in which I found relatively large numbers of high F_{IS} values, and in which such artifacts have been suggested to underestimate heterozygote frequencies; Foltz and Hu, 1996). From this broader phylogenetic perspective, heterozygote deficits in anthozoans and bivalves might be viewed not as consequences of selfing, selection, or segregation of nulls, but of the breeding system.

Fertilization success and the Wahlund effect

Unrecognized population structure (the Wahlund effect) can produce high inbreeding coefficients in the face of wide geographic dispersal of propagules such as planktonic marine larvae (e.g., Johnson and Black, 1982). A potentially powerful mechanism for producing within-population genetic structure in marine animals with dispersing larvae depends on the ecology of fertilization and high variance in individual reproductive success (Hedgecock, 1994; Li and Hedgecock, 1998). Many (but not all) species with dispersing larvae also reproduce by broadcast spawning their gametes into the plankton (Pechenik, 1999). Field studies suggest that fertilization rates for many free-spawners may often be low in general and highly variable among individuals because sperm are short-lived, swim slowly, diffuse quickly, or are not released sufficiently closely to eggs in time or space (Levitan, 1996, 2002; Yund, 2000). These conditions may frequently result in sperm limitation on zygote production. Sperm limitation and frequent fertilization failure for some males may lead to high variance in fertilization success among individuals and, in particular, high variance in paternity rates among males due to the vagaries of fertilization success when sperm are released into the plankton. As a result, single cohorts of offspring produced during one spawning event

could include a small fraction of the alleles present among reproductive adults within the population. If fertilization success is random in a single spawning event with respect to genotype, then cohorts may often differ from each other (Johnson and Black, 1982; Edmands *et al.*, 1996; David *et al.*, 1997; Planes and Lenfant, 2002) depending on the chance combinations of male and female alleles in different spawning events (even for different spawning events in the same group of adult genotypes; Li and Hedgecock, 1998). Finally, age-structured populations may consist of many such genetically differentiated cohorts in each population, with greater heterozygote deficits within populations of older individuals that consist of larger numbers of cohorts (Watts *et al.*, 1990; Edmands *et al.*, 1996). This within-population genetic structure is a type of temporal Wahlund effect that could lead to low frequencies of heterozygotes within populations relative to equilibrium expectations based on equal mating success for all genotypes within the population (Johnson and Black, 1982; Grosberg and Cunningham, 2001). David *et al.* (1997) noted that such a population is "not a defined genetic entity, but rather a part of a genetic mosaic fluctuating in time" under the influence of temporally varying zygote pools that are produced by small adult spawning groups in which individuals vary widely in their contribution of alleles to zygotes.

Exploring the fertilization-inbreeding correlation

My review suggests that the fertilization ecology of marine invertebrates may be a significant force in the genetic structuring of marine invertebrate populations (see Grosberg, 1991). For example, Cohen (1996) compared selfing rates and inbreeding depression for two congeneric tunicate species with free-spawning of sperm and internal or external fertilization of eggs. Cohen (1996) concluded that "site of fertilization may

predict the extent of inbreeding and inbreeding depression in sessile marine invertebrates." Below I discuss some potentially interesting implications of this general result.

Use of genetic markers

Some population genetics research groups that use microsatellite markers commonly discard loci that produce heterozygote deficits in a test population (e.g., Lundy *et al.*, 1999, 2000; Miller *et al.*, 2001; Gold and Turner, 2002). This practice is usually based on the assumption that heterozygote deficits are caused by the presence of unidentified null alleles in apparent homozygote genotypes and on the expectation that single populations will be at equilibrium genotype frequencies. My review suggests that in many cases these departures from Hardy-Weinberg equilibrium genotype frequencies may be caused not by unidentified laboratory artifacts but by population processes (such as high variance in reproductive success or population admixture) that produce a temporal Wahlund effect. Certainly, pedigree analyses of allelic inheritance are needed to confirm the behaviour of microsatellite loci, and any allelic marker that fails to show good Mendelian inheritance in known pedigrees should be treated with caution. However, candidate microsatellite loci that are not at equilibrium in a test population are sometimes discarded in the absence of pedigree information and in spite of the failure to find null homozygotes (an indication that null alleles are rare). Discarding microsatellite markers that are not in Hardy-Weinberg equilibrium on this basis will introduce a potentially significant bias into the resulting estimates of the non-random component of inbreeding. By eliminating loci that may reflect important non-equilibrium processes (such as reproductive variance and temporally varying age structure), this practice may artificially

inflate the relative significance of processes that contribute to random inbreeding (such as dispersal and drift). The tension between equilibrium and non-equilibrium forces in structuring marine invertebrate populations has become an important theme in recent reviews (Grosberg and Cunningham, 2001).

Other studies have used large alignments of mitochondrial DNA sequences to study the spatial distribution and history of genetic differentiation in marine invertebrates with respect to reproductive traits such as dispersal and fertilization (Avise, 1994; Palumbi, 1995; Collin, 2001; Flowers *et al.,* 2002). However, if high variance in paternity rates is an important factor leading to cohort variation and high F_{IS} values at allelic marker loci in many marine invertebrates, then measures of within-population genetic diversity based on haploid maternal markers such as animal mtDNA polymorphisms may not fully reflect the population genetic effects of fertilization processes (Edmands *et al.,* 1996; Flowers *et al.,* 2002). Only paternally or biparentally inherited markers would be expected to reveal the genetic variation among cohorts that should be associated with male broadcast spawning and variance in paternity rates caused by factors associated with fertilization ecology. A combination of the two types of markers may be preferable for studying cohort differentiation and inbreeding, because allelic markers provide estimates of inbreeding coefficients within cohorts while mtDNA sequences provide highly sensitive estimates of among-cohort variation (Chapter Five; Flowers *et al.,* 2002) that may not be detectable using highly polymorphic microsatellites.

Planned comparisons of maternal mitochondrial sequences versus allozyme or microsatellite markers in species with and without free-spawning of sperm are needed to test the hypothesis that variance in paternity rates but not maternity rates is an important influence on among-cohort differentiation and high F_{IS} values. Use of paternal markers

such as the male mitochondrial lineages of some bivalves (Zouros *et al.*, 1994; Stewart *et al.*, 1996) should show large differences in male versus female N_e that are correlated with differences in fertilization ecology and male spawning mode. These differences in propagule dispersal and effective population size based on sex-specific genetic markers are well known in some plants (Mogensen, 1996; Latta and Mitton, 1999) but poorly studied in marine invertebrates.

Paternity rates

Ecological or evolutionary variation in population density (e.g., aggregation of spawning adults), gamete properties (e.g., sperm swimming speed, egg size as a target for sperm), or habitats of adults that differ in oceanographic conditions during spawning (e.g., tide pools versus wave-swept shores) could all lead to variation in fertilization conditions and paternity rates, with consequent differences in genetic variation among cohorts and within populations. More planned comparisons of fertilization variation are needed for populations or species of free-spawners that have well-characterized differences in fertilization kinetics, spawning seasons, or other ecological factors that might affect rates of fertilization and their individual variances (e.g., Levitan, 2002).

More studies are also needed of fertilization, paternity rates, and population genetic variation in species with planktonic sperm and internal fertilization of eggs ('spermcasting'; Pemberton *et al.*, 2003b) in order to test the strength of this association between variance in paternity rates, non-random mating, and inbreeding coefficients. If spermcasting is accompanied by high inbreeding coefficients (as my review suggests), but not by high among-male variance in fertilization success (see Brazeau and Lasker, 1992; Marshall, 2002), then an additional or alternative explanation for this pattern would be

required. Yund (2000) also noted the need for more studies of fertilization ecology in spermcasters. The details of fertilization mechanisms may be crucial to this comparison between spermcasters and free-spawners. For example, some spermcasters can use the adult suspension feeding structure to capture sperm bundles from the plankton, and so might not experience low fertilization rates as a consequences of low sperm availability (see Yund and McCartney, 1994; Atkinson and Yund, 1996; Bishop, 1998; Bishop *et al.*, 2000; Pemberton *et al.*, 2003a).

Conclusions

I found a striking pattern in F_{IS} values with respect to spawning mode: higher inbreeding coefficients are associated with male free-spawning of sperm into the plankton but are not associated with differences in female spawning of eggs. The result suggests that among-species differences in male reproductive ecology can influence the evolution of population genetic structure in marine invertebrates. I propose that high variance in male (but not in female) fertilization success is one sex-biased mechanism documented for broadcast spawning sea urchins that could influence within-population genetic variation among species that differ in male (but not female) reproductive ecology. Additional planned studies of inbreeding coefficients and reproductive success are needed to test other aspects of this hypothesis.

CHAPTER SEVEN

CONCLUSION

I used a series of population genetic studies to characterize variation within and among populations of green sea urchins (*Strongylocentrotus droebachiensis*). I analysed variation at two different classes of genetic markers, on several different spatial scales, and across recruitment events that are a major source of population variation in this species and in many other marine invertebrates.

The first major conclusion from my thesis is that multiple important biological processes influence population genetic variation in this species. Since first arriving in the north Atlantic from the Pacific 3.5 million years ago (Vermeij, 1991), the demography and population genetics of green sea urchins have been influenced by population fluctuations during the Pleistocene Ice Ages, both trans-Arctic and trans-Atlantic dispersal events, hybridization with the congener *S. pallidus*, mass mortality as a result of extensive epizootics, and commercial harvesting for the sushi trade. The most influential of these factors appears to be the oldest: the trans-Arctic re-invasion of sea urchins from Pacific (ancestral) populations approximately 0.9 to 0.4 Mya. Since then, the size and distribution of sea urchin populations in the north Atlantic have probably fluctuated with the Pleistocene glaciations. However, compared to other marine invertebrates in the northwest Atlantic that were extirpated by the most recent glacial maximum (ca. 0.02 Mya), the relatively great age of sea urchin populations estimated from mtDNA haplotype diversity suggests that recent glaciations had a relatively small effect. There has been a unidirectional exchange of genes westward across the north Atlantic, but the

magnitude of this event has had a relatively small impact on the genetic structure of northwest Atlantic populations. Similarly, *S. pallidus* mtDNA haplotypes are strikingly different and illustrate an unexpected source of genetic variation in *S. droebachiensis* populations, but this introgression has had a relatively small impact on overall population genetic variation.

The second major insight from my study is that these sea urchin populations are far from the allele and genotype frequencies predicted under equilibrium conditions. Populations of sea urchins in the northwest Atlantic are still recovering from founding events and other biogeographic influences. Levels of gene flow around the northwest Atlantic appear to be high enough to homogenize allele frequencies among populations within this region. However, patterns of genetic patchiness and high inbreeding coefficients indicate that these populations have yet to achieve genetic equilibrium. Grosberg and Cunningham (2001) point out that many marine species may never achieve genetic equilibrium simply as a result of their large population sizes. To the extent that this is true, measured patterns of genetic structure may not reflect the contemporary magnitude and direction of gene flow, and may largely reflect historical patterns of colonization, range expansion, population size, and alterations in the speed and direction of ocean currents.

Age class analysis revealed a significant amount of temporal variation in allele frequencies within populations. If this variation is caused by a large variance in reproductive success then these patterns of genetic disequilibrium may be a common feature of the green sea urchin and other marine invertebrates with similar breeding systems and dispersal biology. The open nature of sea urchin populations (e.g., local

population dynamics are driven by a supply of recruits from distant populations and not by local reproduction) and the free-spawning of their gametes have resulted in a collection of genetically homogeneous populations in Atlantic Canada that experience a small but significant temporal variation in allele frequencies. Cases such as these have been described as a "genetic mosaic fluctuating in time" (David *et. al.,* 1997). Investigating the causes of such temporal variability may often provide unique insights into the mechanisms that prevent population genetic substructure from approaching equilibrium conditions.

The phylogeographic analysis (Chapter Four) clearly suggests that multiple overlapping processes contribute to population structure. The third conclusion from my study is that high variance in reproductive success (the sweepstakes hypothesis of Hedgecock) could make a significant contribution to non-equilibrium population genetic patterns in this species and perhaps in many others. The following observations from my thesis are consistent with the expected population genetic consequences of sweepstakes reproduction:

- high inbreeding coefficients without significant overall spatial differentiation at microsatellite loci in northwest Atlantic sea urchin populations (Chapter Three)
- patchy, low-magnitude differentiation at microsatellite loci between some pairs of sea urchin populations (Chapter Three)
- significant temporal variation in mtDNA haplotype and microsatellite allele frequencies in analyses of cohorts and year classes (Chapter Five)

- significantly higher mean inbreeding coefficients among species with free-spawn spawning by males compared to species in which males copulate with females (Chapter Six)

One mechanistic process that could produce such population genetic patterns is among-individual variation in reproductive success at fertilization. Field experiments have revealed a considerable amount of variation in fertilization success of individual sea urchins that is correlated with spatial arrangements and density of spawners, water flow, sex ratio, and presence of other species (e.g., Levitan, 1996; 2002). These experiments clearly show the enormous potential for unequal contribution to subsequent generations. The detection of population genetic patterns that are consistent with sweepstakes reproduction in the green sea urchin suggests that it may be a common feature of marine free-spawners. Although the magnitude of the reduction in effective population size relative to the census population size was not characterized in this study, it is clear that this mechanism may be generating the subtle patterns observed in this and other population genetic studies of marine free-spawners.

The literature review (Chapter Six) suggests that differences in fertilization ecology – possibly associated with variance in reproductive success – may consistently contribute to previously neglected sources of genetic variation (i.e., inbreeding coefficients). Greater support for the mechanism must come from improved spatial and temporal sampling in population genetic studies. As outlined in Chapter Six, planned comparisons of population genetics among closely related species with different reproductive ecology will provide better tests of the hypothesis.

The fourth insight from my thesis is illustrated in Chapter Five: the potential for interactions between different processes that can influence non-equilibrium population genetic structure (see Figure 5.3). Specifically, I concluded that population genetic analyses alone are clearly necessary but not sufficient to test the potential influence of variation in reproductive success on population genetic patterns. Age structure within populations could be produced by reproductive variation among individuals or by temporal variation in the source of recruits from genetically differentiated adult spawning populations. Direct observations of reproductive variance may often be necessary in order to measure the specific influence of reproductive variance on age structure and inbreeding levels (the temporal Wahlund effect). Without such observations, the insights from population genetic patterns may only be consistent with (but not direct demonstrations of) the population genetic consequences of sweepstakes reproduction.

Many of these general conclusions depended on the use of two different classes of genetic markers in the same population samples. The fifth insight from my thesis is the value of combining population genetic analyses that use allelic markers from the nuclear genome with sequence data from the mitochondrial genome. Microsatellites had many advantages over mtDNA sequences. They provided multiple estimates of population structure at unlinked loci, and because microsatellite genotyping is cheap and fast, I was able to include a large number of individuals in the analyses. Both these factors (multiple loci and large sample size) improved the estimation of population substructure. Microsatellites also provided measures of high inbreeding coefficients in northwest Atlantic populations; this observation motivated many of the other studies including the literature review and analysis of age structure. One disadvantage of using microsatellites

was that they were generally too variable to resolve much of the temporal structure (Chapter Five). Microsatellites were also too variable to detect genetic structure on large spatial scales: one locus in European populations was fixed for one or more null alleles (Chapter Three). This suggests that many microsatellite markers may be unsuitable for analyses of population structure in species with complex, old histories of range expansion and population isolation (such as the parallel invasions of the eastern and western Atlantic). Microsatellite markers also failed to reveal the introgression of *S. pallidus* markers into *S. droebacheinsis* populations (Chapter Four) because all available *S. pallidus* specimens were fixed for null alleles at all four microsatellite loci. The final disadvantage of using microsatellite markers in my studies of sea urchins is that, although the among ocean comparisons were consistent with the mtDNA analysis, they provided much less resolution of historical relationships among populations.

The main advantage of using mtDNA sequences in my studies of sea urchins was that the temporal information in the gene genealogy revealed the historical relationships among populations on a large spatial scale. Of particular interest was the pattern of population expansion across the Arctic and the subsequent genetic diversification (Chapter Four). This relationship was not detectable from the patterns of genetic variation in the microsatellite markers (Chapter Three). Mitochondrial DNA also provided a more sensitive estimate of age structure (Chapter Five). One disadvantage of using mtDNA markers was that they are relatively more expensive and more labour intensive than microsatellites. Finally, since mtDNA is maternally inherited, variation in haplotype frequencies were insensitive to the influence of variance in male reproductive success (Chapter Five) suggested by Chapter Six.

The advantages and disadvantages of these two classes of markers are well known to population geneticists. Nevertheless, most previous studies of sea urchin population genetic variation have focused on just allelic markers such as microsatellites, allozymes, and nuclear DNA sequences (Marcus, 1977; Watts *et. al.,* 1990; Mladenov *et. al.,* 1997; Debenham et al., 2000; Moberg and Burton 2000) or just mtDNA sequences (Palumbi and Wilson, 1990; Palumbi and Kessing, 1991; McCartney *et. al.,* 2000; Flowers *et. al.,* 2002; Lessios *et. al.,* 1998, 1999, 2001, 2003). Only one study combined these two types of markers (Edmands *et. al.,* 1996). The potential advantages of combining these two classes of markers are clear in practice: the microsatellite analyses of inbreeding coefficients could not have been obtained from haploid maternal sequence markers, and these surprisingly high inbreeding coefficients motivated much of the rest of my thesis work, but the mtDNA sequences were generally more effective at identifying temporal and spatial structure within the same population samples.

As a discipline, marine population genetics has been intensively focused on the study of larval dispersal and the genetic effects of ecological or evolutionary variation in dispersal potential. It is easy to understand the attraction to these relationships: the ocean is a nurturing environment (compared to airborne dispersal of terrestrial organisms) in which dispersing propagules like larvae can develop (Strathmann, 1990), and marine invertebrates vary tremendously in the dispersal potential of these propagules because some do and some do not develop as larvae in the plankton. Since this difference in the habitat for larval development is correlated with dispersal ability and numerous other reproductive and early life history traits (Pechenik, 1999; Strathmann, 1985), the

correlated evolution of early life history traits and dispersal ability has also become the focus of population genetic analysis.

The final conclusion of my thesis is that this focus should probably change. Population genetic studies of dispersal variation generally assume a dynamic equilibrium between gene flow and genetic differentiation among populations. However, many studies now suggest that equilibrium dynamics should not be expected in many marine species, and there are many exceptions to the predicted negative correlation between dispersal potential and population differentiation. For many of these studies there is often insufficient behavioural, ecological, or biogeographical information to adequately discriminate among the various mechanisms that could generate the observed patterns of population genetic structure and departure from equilibrium dynamics. Studies like mine, which highlight the potential significance of biogeographical history and fertilization ecology (among other effects), suggest that more marine population genetics studies should focus on the effects of these non-equilibrium processes on genetic variation within and between populations, especially in taxa where the dispersal-differentiation correlation appears to be weak. This new focus on non-equilibrium dynamics should aim to advance our understanding of the mechanisms that generate small-scale temporal and spatial genetic structure. To do this it will be necessary to extend traditional population genetic studies to include analyses of age class structure, and wherever possible analyses of genetic variation in early life history stages.

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Appendix 1. Summary Information for the Literature Review in Chapter 6.

Each entry includes taxonomic information, 5-letter codes for reproductive traits, average population sample size, F_{IS} value (averaged across loci), and abbreviated reference. Reproductive traits and abbreviations are male broadcast spawning (B), pseudocopulation (P), or copulation (C); female broadcast spawning (S), egg masses (M), or internal fertilization (I); larval dispersal in the plankton (K), limited dispersal (L), or no planktonic larval stage (N); with a significant mode of asexual reproduction (A) or sexual only (X); dioecious breeding system (D) or some form of hermaphroditism (H); unknown reproductive traits are shown by ?. Table entries are sorted alphabetically by genus name within each phylum.

Cnidaria

coral	*Acropora cuneata*	BILA?	45.4	0.2700	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Acropora cytherea*	BSKX?	48.8	0.1300	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Acropora hyacinthus*	BSKXH	48.6	0.2500	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Acropora millepora*	BSKAH	48.3	0.3700	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Acropora palifera*	BILA?	47	0.2200	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Acropora valida*	BSKA?	44.2	0.3500	Ayre & Hughes 2000 Evolution 54, 1590

anemone	*Anthopleura artemisia*	BSKAD	20	0.5970	Smith & Potts 1987 Mar. Biol. 94, 537
anemone	*Anthopleura elegantissima*	BSKXD	39.6	0.1250	McFadden et al. 1997 Mar. Biol. 128, 127
anemone	*Anthopleura elegantissima*	BSKAD	30.2	0.1340	McFadden et al. 1997 Mar. Biol. 128, 127
anemone	*Anthopleura elegantissima*	BSKAD	22.6	0.1980	Edmands & Potts 1997 Mar. Biol. 127, 485
anemone	*Anthopleura elegantissima*	BSKXD	20	0.4870	Smith & Potts 1987 Mar. Biol. 94, 537
anemone	*Anthopleura elegantissima*	BSKAD	20	0.5180	Smith & Potts 1987 Mar. Biol. 94, 537
anemone	*Anthopleura xanthogrammica*	BSKXD	20	0.4840	Smith & Potts 1987 Mar. Biol. 94, 537
black coral	*Antipathes fiordensis*	BSKXD	27	0.3130	Miller 1997 Mar. Ecol. Prog. Ser. 161, 123
cup coral	*Balanophyllia elegans*	BINXD	?	0.0210	Hellberg 1994 Evolution 48, 1829
cup coral	*Balanophyllia elegans*	BINXD	32	0.0306	Hellberg 1996 Evolution 50, 1167
octocoral	*Clavularia koellikeri*	BIKAD	40.5	0.0280	Bastidas et al. 2002 Coral Reefs 21, 23
anemone	*Epiactis lisbethae*	BINXD	12.1	0.9570	Edmands & Potts 1997 Mar. Biol. 127, 485

anemone	*Epiactis prolifera*	BINAH	19.5	0.9950	Edmands & Potts 1997 Mar. Biol. 127, 485
anemone	*Epiactis ritteri*	BINXD	13	0.8310	Edmands & Potts 1997 Mar. Biol. 127, 485
coral	*Paracyathus stearnsii*	BSKXD	36.8	-0.0021	Hellberg 1996 Evolution 50, 116
coral	*Platygyra sinensis*	BSKAH	33	0.0750	Ng & Morton 2003 Mar. Biol., in press
coral	*Pocillopora damicornis*	BILAH	45.2	0.2800	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Pocillopora verrucosa*	BSKXD	44.5	0.5191	Ridgway et al. 2001 Mar. Biol. 139, 175
coral	*Seriatopora hystrix*	BILAH	38.3	0.2300	Ayre & Duffy 1994 Evolution 48, 1183
coral	*Seriatopora hystrix*	BILAH	45.3	0.5100	Ayre & Hughes 2000 Evolution 54, 1590
coral	*Seriatopora pistillata*	BILXH	45.7	0.1700	Ayre & Hughes 2000 Evolution 54, 1590
soft coral	*Sinularia flexibilis*	BSKAD	41	0.0343	Bastidas et al. 2001 Mar. Biol. 138, 517
zoanthid	*Zoanthus coppingeri*	BSKA?	63.8	0.1270	Burnett et al. 1995 Mar. Biol. 122, 665
Echinodermata					
seastar	*Acanthaster planci*	BSKXD	45	0.0720	Benzie & Stoddart 1992 Mar. Biol. 112, 119

seastar	*Acanthaster planci*	BSKXD	28	0.0850	Benzie & Stoddart 1992 Mar. Biol. 112, 119
seastar	*Acanthaster planci*	BSKXD	?	0.1566	Nash et al. 1988 Coral Reefs 7, 11
seastar	*Coscinasterias muricata*	BSKXD	36.7	0.0810	Skold et al. 2003 Mar. Ecol. Prog. Ser. 250, 163
seastar	*Evasterias troschelii*	BSKXD	96.5	0.2470	Stickle et al. 1992 Can. J. Zool. 70, 723
sea urchin	*Evechinus chloroticus*	BSKXD	42.8	0.0500	Mladenov et al. 1997 N.Z.J.Mar.Freshw. Res. 31,261
sea cucumber	*Holothuria atra*	BSKAD	50	-0.0780	Uthicke et al. 2001 Mar. Biol. 139, 257
sea cucumber	*Holothuria atra*	BSKAD	77.8	-0.0740	Uthicke et al. 1998 Mar. Biol. 132, 141
sea cucumber	*Holothuria nobilis*	BSKXD	57.1	0.0628	Uthicke & Benzie 2000 Mar. Biol. 137, 819
seastar	*Leptasterias epichlora*	PENXD	86	0.0080	Stickle et al. 1992 Can. J. Zool. 70, 723
seastar	*Leptasterias epichlora*	PENXD	55	0.1240	Kwast et al. 1990 Mar. Biol. 105, 477
seastar	*Leptasterias hexactis*	PENXD	69	0.0450	Stickle et al. 1992 Can. J. Zool. 70, 723

seastar	*Leptasterias hexactis*	PENXD	11.5	0.3110	Kwast et al. 1990 Mar. Biol. 105, 477
seastar	*Leptasterias polaris*	PENXD	60	-0.0300	Stickle et al. 1992 Can. J. Zool. 70, 723
seastar	*Linckia laevigata*	BSKXD	100	0.0421	Williams & Benzie 1993 Mar. Biol. 117, 71
seastar	*Linckia laevigata*	BSKXD	27.5	0.0669	Juinio-Meñez et al. 2003 Mar. Biol. 142, 717
seastar	*Patiriella calcar*	BSKXD	38	0.0650	Hunt 1993 Mar. Ecol. Prog. Ser. 92, 179
seastar	*Patiriella exigua*	PENXH	41	0.0980	Hunt 1993 Mar. Ecol. Prog. Ser. 92, 179
seastar	*Pisaster ochraceus*	BSKXD	107.3	0.0880	Stickle et al. 1992 Can. J. Zool. 70, 723
sea cucumber	*Stichopus chloronotus*	BSKAD	51.6	-0.5360	Uthicke et al. 2001 Mar. Biol. 139, 257
sea urchin	*Strongylocentrotus droebachiensis*	BSKXD	87	0.1430	Addison & Hart 2004 Mar. Biol., 144, 243
Crustacea					
amphipod	*Abyssorchomene* sp.	CILXD	113.4	0.1600	France 1994 Mar. Biol. 118, 67

shrimp	*Artemesia longinaris*	CIKXD	107	-0.0570	Weber et al. 1993 Comp. Biochem. Physiol. 106B4, 1015
ostracod	*Cyprideis torosa*	CI?XD	117.3	0.1115	Sywula et al. 1995 Mar. Biol. 121, 647
shrimp	*Litopenaeus setiferus*	CIKXD	79.5	0.131	Ball & Chapman 2003 Mol. Ecol. 12 2319
prawn	*Penaeus monodon*	CIKXD	62.4	0.0093	Brooker et al. 2000 Mar. Biol. 136, 149
prawn	*Penaeus monodon*	CIKXD	70.5	0.0820	Xu et al. 2001 Aquaculture 199, 13
shrimp	*Rimicaris exoculata*	CIKXD	104.5	0.0530	Creasey et al. 1996 Mar. Biol. 125, 473
barnacle	*Tetraclita squamosa*	CIKXH	66.2	0.1200	Ford & Mitton 1993 Mol. Mar. Biol. Biotech. 2, 147
fiddler crab	*Uca arcuata*	CIKXD	29.7	0.2630	Huang & Shih 199[illegible] Hydrobiologia 295, 67
amphipod	*Ventiella sulfuris*	CINXD	67	0.1960	France et al. 1992 Mar. Biol. 114, 551
Mollusca					
nudibranch	*Adalaria proxima*	CIKXH	47	0.000	Todd et al. 1998 J. Exp. Mar. Biol. Ecol. 228, 1

cockle	*Anomalocardia brasiliana*	BSKXD	112.8	0.1480	da Silva & Sole-Cava 1994 Genetic: Evol. Aquat. Org.
sea hare	*Aplysia californica*	CIKXH	35.4	0.0789	Medina & Walsh 2000 Mar. Biotechnol. 2, 449
mussel	*Bathymodiolus thermophilus*	BSKXD	35.6	0.1370	Craddock et al. 1995 Mar. Biol. 124, 137
whelk	*Bullia digitalis*	CINXD	87.6	0.0100	Grant & da Silva-Tatley 1997 Mar. Biol. 129, 123
cockle	*Cerastoderma edule*	BSKXD	100	0.0900	Beaumont & Pethe 1996 Fisheries Res. 28, 263
cockle	*Cerastoderma glaucum*	BSKXD	24.6	0.4390	Mariani et al. 2002 Mar. Biol. 140, 687
slipper shell	*Crepidula fornicata*	CIKXH	50	0.0660	Dupont et al. 2003 Mar. Ecol. Prog. Ser. 253, 183
nudibranch	*Goniodoris nodosa*	CILXH	46.3	0.0060	Todd et al. 1998 J. Exp. Mar. Biol. Ecol. 228, 1
black abalone	*Haliotis cracherodii*	BSKXD	?	0.1250	Hamm & Burton 2000 J. Exp. Mar. Biol. Ecol. 254, 23
abalone	*Haliotis fulgens*	BSKXD	20.4	0.3180	Zuniga et al. 2000 J. Shellfish. Res. 19, 853

abalone	*Haliotis rubra*	BSKXD	84	0.0570	Brown 1991 Aust. J. Mar Freshwater Res. 42, 77
abalone	*Haliotis rubra*	BSKXD	10	0.6347	Huang et al. 2000 Mar. Biol. 136, 207
periwinkle	*Littorina mariae*	CINXD	80.5	0.0980	Rolan-Alvarez 1995 Heredity 74, 1
periwinkle	*Littorina obtusata*	CINXD	262	0.0140	Rolan-Alvarez 1995 Heredity 74, 1
periwinkle	*Littorina saxatilis*	CINXD	34.5	0.0700	Jansen & Ward 1982 Biol. J. Linn. Soc. 22, 289
mussel	*Mytilus edulis*	BSKXD	1000	0.0270	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225
mussel	*Mytilus edulis*	BSKXD	159	0.0650	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225
mussel	*Mytilus edulis*	BSKXD	1000	0.0920	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225
mussel	*Mytilus edulis*	BSKXD	?	0.1280	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225
mussel	*Mytilus edulis*	BSKXD	107.3	0.1620	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225
mussel	*Mytilus galloprovincialis*	BSKXD	24	0.0500	Raymond et al. 1997 Mar. Ecol. Prog. Ser. 156, 225

mussel	*Mytilus galloprovincialis*	BSKXD	50	0.0900	Skalamera et al. 1999 Mar. Ecol. Prog. Ser. 178, 251
mussel	*Mytilus galloprovincialis*	BSKXD	40	0.1023	Daguin & Borsa 1999 J. Exp. Mar. Biol. Ecol. 235, 55
whelk	*Nucella emarginata*	CINXD	25	0.2061	Marko 1998 Evolution 52, 757
whelk	*Nucella lamellosa*	CINXD	76.2	0.0860	Grant & Utter 1988 Malacologia 28, 275
whelk	*Nucella ostrina*	CINXD	25	0.0639	Marko 1998 Evolution 52, 757
octopus	*Pareledone turqueti*	CIKXD	120	0.0690	Allcock et al. 1997 Mar. Biol. 129, 97
scallop	*Pecten jacobaeus*	BSKAH	10	0.2840	Rios et al. 2002 J. Exp. Mar. Biol. Ecol. 267, 223
scallop	*Pecten maximus*	BSKAH	7	0.3070	Rios et al. 2002 J. Exp. Mar. Biol. Ecol. 267, 223
clam	*Ruditapes decussatus*	BSKXD	92.4	0.0600	Borsa et al. 1991 Heredity 66, 1
cuttlefish	*Sepia officinalis*	CINXD	27.6	0.0160	Perez-Losada et al. 1999 Heredity 83, 280
limpet	*Siphonaria kurracheensis*	CIKXH	?	0.1050	Johnson et al. 2001 Mar. Biol. 139, 108
whelk	*Stramonita haemostoma*	CIKXD	139.2	0.0180	Liu et al. 1991 Mar Biol. 111, 71

whelk	*Stramonita haemostoma*	CIKXD	67.3	0.0790	Liu et al. 1991 Mar Biol. 111, 71
snail (topshell)	*Tectus coerulescens*	B?KXD	20	0.1441	Borsa & Benzie 1996 Mar. Biol. 125, 531
giant clam	*Tridacna crocea*	BSKXD	26.7	0.2763	Juinio-Meñez et al. 2003 Mar. Biol. 142, 717
giant clam	*Tridacna derasa*	BSKXD	48.7	0.0410	Macaranas et al. 1992 Mar. Biol. 113, 231
giant clam	*Tridacna gigas*	BSKXD	33.5	0.0250	Benzie & Williams 1992 Mar. Biol. 113, 373
giant clam	*Tridacna gigas*	BSKXD	69	0.0280	Benzie & Williams 1995 Mar. Biol. 123, 781
giant clam	*Tridacna maxima*	BSKXD	18.1	0.0540	Benzie & Williams 1992 Coral Reefs 11, 135
snail (topshell)	*Trochus niloticus*	BSKXD	68	0.1063	Borsa & Benzie 1996 Mar. Biol. 125, 531
clam	*Venus antiqua*	BSKXD	28.8	0.5700	Gallardo et al. 1998 J. Exp. Mar. Biol. Ecol. 230, 193
Annelida					
ice cream cone worm	*Pectinaria koreni*	BSKXD	29.6	0.341	Jolly et al. 2003 Helgol. Mar. Res. 56, 238

vent worm	*Riftia pachyptila*	BSKXD	38.6	0.0560	Black et al. 1994 Mar. Biol. 120, 33
Urochordata					
tunicate	*Botryllus schlosseri*	BILAH	24	0.2828	Stoner et al. 2002 Mar. Ecol. Prog. Ser. 243, 93
tunicate	*Botryllus schlosseri*	BILAH	50	0.3380	Grosberg 1991 Evolution 45, 130
Nemertea					
nemertean	*Parbolasia corrugatus*	BSKXD	?	0.2400	Rogers et al. 1998 Mar. Biol. 131, 1

Appendix 2 Allelic richness for populations of sea urchins (*Strongylocentrotus droebachiensis*) calculated based on a random sample of 37 diploid individuals using FSTAT (Goudet, 2001). Population abbreviations are the same as those reported in Table 3.1 with the exception of SJC (San Juan Channel, Pacific Ocean), NOR (Vestfjorden, Norway), and ICE (Hvalfjordur, Iceland). NA indicates that locus *Sd*76 failed to amplify in these populations.

	Locus			
Population	*Sd*156	*Sd*121	*Sd*63	*Sd*76
SJC	13.00	16.79	11.77	12.00
CBY	13.30	16.28	10.04	12.11
BBY	12.01	16.35	11.10	13.05
HSP	10.57	17.23	10.20	11.54
MBY	11.90	18.68	12.32	12.33
MAD	11.65	15.39	11.07	11.47
JED	11.47	17.96	11.44	12.47
HFX	11.30	13.91	8.84	11.60
BCV	11.94	15.24	12.29	11.94
DBY	11.07	16.26	11.50	12.53
ISH	13.08	17.61	13.22	12.91
NOR	3.87	6.31	5.41	NA
ICE	3.70	6.50	7.19	NA