# LIFE HISTORY AND POPULATION GENETIC STRUCTURE OF SEA STARS FROM THE FAMILY ASTERINIDAE

by

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**Life history and population genetic structure of sea stars from the family Asterinidae.**

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# ABSTRACT

Life history can influence population genetic variation by altering patterns of gamete union and dispersal. Sea stars from the family Asterinidae have evolved similar life histories multiple times in parallel including planktonic feeding larvae, planktonic non-feeding larvae, development in benthic egg masses, and viviparity. In this thesis I first examine the population genetic structure of a widespread planktotrophic asterinid sea star from the East Pacific (*Patiria miniata).* I use mitochondrial sequence markers to determine whether extrinsic factors such as vicariance or intrinsic properties such as dispersal mode are driving patterns of population genetic variation in this species. I then examine patterns of population genetic variation among eight additional asterinid species from Australia using a mixed species pool of genomic microsatellite markers. I use microsatellite markers to characterize the genetic variation within groups of brooded offspring associated with the unusual life histories of two live bearing asterinids from the genus *Parvulastra.* Lastly, I examine the evolution of life history among asterinids and use the phylogenetic relationships among species to examine the correlation between life history and population genetic structure in this group. Ultimately, I find that the degree to which intrinsic life history properties of asterinids and extrinsic factors contribute to population genetic

variation varies among species and among clades. In *P. miniata* patterns of population genetic variation are influenced by both intrinsic and extrinsic factors. Using microsatellite markers I find that in general between-population genetic variation is high in benthic species (benthic egg laying and live bearing) relative to species with planktonic larvae and that genetic variation within populations is lower in benthic species relative to planktonic species. Lastly, I find that the degree to which phylogeny constrains the coevolution of population genetic structure and life history varies among life history characters and among-population genetic parameters. This thesis suggests that in many cases variation in life histories among a closely related group of marine species can predict patterns of population genetic variation. However, extrinsic factors can in some cases, act with or override life history characteristics in driving patterns of population genetic variation.

**Keywords:** life history, Asterinidae, population genetics, microsatellite marker, comparative method, mating system, brooding

# DEDICATION

I would like to dedicate this thesis to Lindy and John Keever, who are the most wonderful parents a girl could ask for, and who have made it possible for me to achieve my goals.

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# Chapter 1 **GENERAL INTRODUCTION**

Life history can modify population genetic structure by driving patterns of gamete union and postzygotic dispersal. These patterns impact higher-order processes such as local adaptation, speciation, and extinction (Williams and Benzie 1998; Benzie and Williams 1997; Palumbi 1997). The life history variables, including reproductive and dispersive factors, influencing population genetic structure in marine invertebrates include adult fecundity, fertilization behaviour, hermaphroditism, brood protection (or lack thereof), and larval dispersal (Strathmann 1985; Knowlton and Jackson 1993). Most previous studies of reproductive variation and population genetic structure have used species or higher taxon comparisons that did not identify the population genetic similarities that arise due to shared ancestral similarities in reproduction among members of the same clade, or convergent similarities evolved as a consequence of parallel adaptive changes in reproduction in separate clades (Collin 2001). My graduate research examines associations between life history and population genetic structure among closely related asterinid sea stars, and applies well-known phylogenetic relationships among those species to distinguish shared ancestry from adaptive life history evolution as likely causes of variation in population genetic structure.

I focus on two life history traits: the mode of larval development and the mode of fertilization. Like other benthic marine species, asterinids with a planktonic larval phase are usually expected to have decreased levels of genetic differentiation on account of high migration between populations. The presence of a planktonic larval phase may also be positively correlated with levels of allelic diversity and heterozygosity within populations because high gene flow can slow the loss of alleles due to genetic drift (Pechenik 1999). Similarly, asterinids that have planktonic fertilization, large adult body size, and high fecundity are expected to have high heterozygosity and large effective population sizes compared to species with benthic fertilization (with small adults and low fecundity) (Cohen 1996, Ayre et al 1997, Addison and Hart 2003). In addition, some species with benthic fertilization are also simultaneous hermaphrodites, in which self-fertilization is expected to lead to local inbreeding, strong genetic drift, loss of within-population genetic diversity, and substantial between-population differentiation.

Comparative studies of closely related species have found that life history traits vary in their influence on population genetic structure. Population genetic research suggests that in many cases larval dispersal mode (Duffy 1993, Hunt 1993, Hoskin 1997, Lee and Boulding 2009) or mating system variation (Edmands and Pots 1997) predicts levels of within- and between-population genetic variation. Often life history influences the way that a population responds to extrinsic forces such as historical vicariance (Hellberg 1996, Arndt and Smith 1998, Bohonak 1999, Hellberg et al. 2002). However, numerous counter

examples have found that historical biogeographic events, variable current patterns and differences in other aspects of life history such as ecological niche can override the influences of larval form and mating system in predicting similarities or differences in population genetic variation among closely related species (McMillan et al. 1992, Burton 1997, Kyle and Boulding 2000, Hickerson and Cunningham 2005, Marko 2004, Ayre et al. 2009).

More recent broad taxonomic studies of marker type, geographic location, taxonomy and life history have shown an association with levels of genetic variation. An analysis by Bradbury et al. (2008) found a significant association between taxonomic rank, geography, and dispersal mode as predictors for population genetic subdivision. Another study by Weirsing and Toonen (2008) found that there was a strong relationship between planktonic larval duration and population genetic differentiation measured as $F_{st}$, but that this pattern broke down when species with completely non-planktonic larvae were removed from the analysis.

A handful of comparative studies of a broad taxonomic array of sympatric species in specific biogeographic regions suggest that historical factors and ecological associations may be more important than life history in predicting levels of population genetic variation (Teske et al. 2007, Ayre et al. 2009, Marko et al. 2009). These studies, although extensive, would benefit from increased sample size, and a broader sample of species with diverse life histories will bring me closer to confidently inferring the relative role of life history on population genetic structure in marine species.

In this thesis, I attempt to build upon the body of research examining correlations between life history and population genetic structure, using closely related species within the family Asterinidae.

## .Study Group

Sea stars of the Family Asterinidae possess a great diversity of larval forms and life histories (Dartnall 1965, Dartnall 1971, Keough and Dartnall 1978 Byrne 1996, Byrne 2006), including **planktotrophy** (feeding larva, which is the ancestral state for the Class Asteroidea), **lecithotroph**y (a non-feeding shorter-lived plantktonic larva), embryos in **benthic egg masses**, and **internal brooding** (some with sibling brood cannibalism). The Pacific asterinids have independently evolved all three derived life history types, some multiple times in parallel (Hart 1997) (see phylogeny in Figure 1.1). An important aspect of this family is that closely related and morphologically similar species will often have major differences in life history mode (Byrne 2003). Similar cases are found among other groups of species, for example gastropods in the genus *Conus* (Duda and Palumbi 1999), *Littorina* (Kyle and Boulding 2000), *Crepidula* (Collin 2001) and echinoids in the genus *Heliocidaris* (McMillan et al. 1992).

Planktotrophic species studied in this thesis include *Meridiastra mortenseni, Patiria miniata,* and *Patiria pectinifera.* These species have large, dioecious adults with high fecundity, small eggs, broadcast spawning, planktonic fertilization, and a morphologically complex feeding (planktotrophic) bipinnaria larva with prolonged planktonic development and growth followed by radical

metamorphosis (Byrne and Barker 1991). Planktotrophy is widely assumed to be plesiomorphic for asterinids and other sea stars (Chia and Walker 1991) because the larval form is morphologically complex and shared among sea star orders (and similar to planktotrophic larvae of other echinoderm classes and some hemichordate worms).

Lecithotrophic species include *Meridiastra calcar* and *Cryptasterina pentagona.* These species spawn eggs and sperm into the plankton where fertilization occurs. Individuals have energetically expensive yolk-rich eggs, smaller clutch size compared to planktotrophs, non-feeding (lecithotrophic) larval morphology and faster planktonic development (Byrne 1992). These large non-feeding larvae lack the complex morphological, physiological, and behavioural adaptations of planktotrophic larvae for suspension feeding and growth. Larval morphology of lecithotrophic species differs between clades, suggesting that a lecithotrophic larva has evolved multiple times in the Asterinidae (Byrne 2006).

*Parvulastra exigua* is the only species in this thesis with development in benthic external egg masses. The life history of this species includes small adults that lay clutches of eggs in sticky benthic masses, with external fertilization by pseudo copulation, and larval development without a planktonic stage (Lawson-Kerr and Anderson 1978; Byrne 1995). Adults change sex from male (when small) to female (when larger), but few individuals of intermediate size have both testes and ovaries so self-fertilization is probably rare and outcrossing is expected to be the usual mode of fertilization. In *P. exigua* and some other

species, ‘females’ abandon the egg mass after fertilization, but other species provide brood care (Emson and Crump 1976, 1984).

Finally, I studied three live-bearing species from two asterinid clades including *Parvulastra vivipara, Parvulastra parvivipara,* and *Cryptasterina hystera.* This life history includes small simultaneous hermaphrodites with internal fertilization (possibly selfing) of eggs, intragonadal brooding, internal metamorphosis, and live birth (or viviparity) of juvenile sea stars (Byrne and Cerra 1996, Byrne 2008). Brooded *Cryptasterina* larvae resemble those of planktonic lecithotrophs. Some *Parvulastra* cannibalize juvenile broodmates before birth (Byrne 1996a, 2006). Brooding *Parvulastra* species have eggs smaller than those of planktotrophs, and develop rapidly into juveniles, followed by extensive brood cannibalism and prolonged growth into very large juveniles before birth.

## .Sampling and Species Ranges

A graphic of sampling locations of all asterinid species used in this analysis and described in the previous section can be found in Figure 1.2, and a reference to each species sampled in this thesis, their ranges, life histories and sampling locations can be found in Appendix 1. Many of the asterinid species sampled in this thesis are native to the continent of Australia. Some species (*P. parvivipara*, and *C. hystera*) are endemic to highly localized regions of Australia or Tasmania, while other species (*M. calcar or P. regularis*) are widespread throughout Australia and New Zealand. Although the Family Asterinidae is a

cosmopolitan group, none of the species sampled in this thesis are found in multiple hemispheres or multiple oceans.

Sampling ranged from a few hundred meters to a few thousand kilometres. With the exception of *Meridiastra mortenseni,* the species with relatively large ranges were sampled at short intervals comparable to those species with small endemic ranges. In most cases the sampled range is highly representative of the species range size. One exception is *P. pectinifera* sampled from the South Island of Japan. This species is broadly distributed throughout the Pacific in Russia and Japan, but was only sampled from a 16-kilometre stretch of coastline on the northern end of the Japanese island of Honshu.

The planktotroph *Patiria miniata* is native to the intertidal region of the northeast Pacific Ocean and ranges from Baja California to southeast Alaska (Kozloff 1983, Lambert 2000). Between 20 and 50 individuals were sampled from 13 populations ranging from Santa Barbara (California) to View Cove (Alaska).

The planktotroph *Patiria pectinifera* is the sister taxon of *P. miniata*, but is native to the intertidal regions of the northwest Pacific Ocean (O'Laughlin and Waters 2004). It is a common species in the intertidal and subtidal throughout Japan and into the Sea of Okhotsk and the Kuril Islands (Kashenko 2005). *P. pectinifera* was sampled from three populations on the northwest corner of the south Island of Japan and the sampling range was on the order of 16 kilometres.

With the help of collaborators, I sampled a third planktotrophic species *Meridiastra mortenseni* in New Zealand. This species was recently described by O'Loughlin (2002), and the known extent of its range includes the north and south islands of New Zealand and the Stewart and Chatham Islands. I sampled 3 populations from the north island of New Zealand. Much of the phylogeographic structure reported in a sympatric planktotroph *P. regularis* (Waters and Roy 2004) was between populations on the northern and southern islands of New Zealand, suggesting that my sampling may miss a major phylogeographic discontinuity in this species.

*Meridiastra calcar* is a lecithotrophic asterinid with a broad distribution spanning southern and eastern Australia and Tasmania (Byrne 1992). This species is found in the mid to low intertidal regions on moderately exposed rocky shores. In total 12 *Meridiastra calcar* populations were sampled throughout a large proportion of this range.

*Cryptasterina pentagona* is the second lecithotrophic species sampled in this analysis. This species ranges from Central Queensland Australia to the Torres Straight in Papua New Guinea and may be more broadly distributed throughout the Indo-Pacific (Dartnall 1971, Marsh 1977, and Dartnall et al 2003). I sampled 6 Australian populations separated by up to about 600 kilometres of coastline between Arlie Beach and Bingal Bay in Queensland.

*Parvulastra exigua* has a similar range to that of lecithotrophic *Meridiastra calcar* however the range of this benthic egg mass depositing species does not

extend as far westward through South Australia as *M. calcar* (Byrne 1992). In total *P. exigua* was sampled form 11 populations, and all but 2 (Port Hughes and Tikera) are overlapping with samples of *Meridiastra Calcar.*

*Cryptasterina hystera*, the live bearing sister species to *C. pentagona,* is highly localized and may be endemic to a small number of sites in southern Queensland including One Tree Island on the Southern Great Barrier Reef and Bargara and Kinka Beaches near Yeppoon in central Queensland (Dartnall et al. 2003, Hart et al. 2003, Byrne et al. 2003). *C. hystera* was sampled from four populations on the Southern Great Barrier Reef at One Tree Island and two populations near Yeppoon on the mainland of Queensland Australia.

Live bearing *Parvulastra parvivipara* is endemic to the Eyre Peninsula in South Australia and is the smallest asterinid with maximum adult size from about 3 to 7 millimetres (Keough and Dartnall 1978). *P. parvivipara* was sampled from four sites spanning 120 kilometres of the western Eyre Peninsula and western South Australia.

*Parvulastra vivipara,* the live bearing sister species of *P. parvivipara,* is endemic to the east coast of Tasmania (Dartnall 1965, Prestedge 1998). This species was sampled from three populations along a 25-kilometre stretch of coastline from Eaglehawk Neck to Fortescue Bay. Tissue samples from *P. vivipara* were collected prior to the sampling date of the other asterinid species (Appendix 1), because collection of this species is now restricted due to its threatened status (TSSG Australia 2009).

## .Hypotheses

Asterinid sea stars provide an excellent opportunity to address the role of life history in population genetic structure (Byrne 2003). The goal of this research is to use nuclear (microsatellites) and mitochondrial DNA markers to evaluate the relative role of life history mode in population genetic structure of species within the family Asterinidae.

If aspects of asterinid life history, including differences in the habitat for development and the mode of fertilization, significantly influence levels of within and between population genetic variation then

1. species with longer planktonic larval durations are predicted to have lower levels of genetic differentiation than species with benthic or internal larval development, and
2. species with gonochoric mating systems with high rates of recombination between gametes should have higher levels of within-population genetic diversity relative to self-fertilizing hermaphroditic or asexual species.

Furthermore, if life history is a strong predictor of patterns of population genetic variation then these effects should be apparent both between species that evolved similar life histories in parallel and species that share a given life history as a plesiomorphic character state.

Rejection of these predictions could have several different causes. First, stochastic processes associated with broadcast spawning could lead to higher levels of local inbreeding relative to those species with internal fertilization or

pseudo copulation (Addison and Hart 2005). Second, historical factors such as changing current patterns, sea level changes, and glaciations are known to have a profound influence on patterns of population genetic variation and geographic structure (Hewitt 2004, Grosberg and Cunningham 2001). If widespread Asterinidae have been strongly influenced by historical vicariance, then population genetic differentiation could be greater then predicted based solely on life history. Lastly, there is a growing speculation that specific habitat preferences and ecological and oceanographic changes among regions can lead to differences in population genetic structure not predicted by life history characteristics alone (Kinlan et al. 2005, Ayre et al. 2009).

## .Thesis Overview

In this thesis I begin by assessing the population genetic structure of a broadly distributed planktotrophic species. Secondly, I qualitatively evaluate the patterns of population genetic structure among closely related asterinid species. I then evaluate the role of mating systems of live bearing asterinids in shaping population genetic structure. Lastly, I conduct a series of quantitative comparative analyses examining the relative role of life history in population genetic structure.

Ch2: I use the widespread and common North American bat star *Patiria miniata* to test the prediction that a species with planktonic feeding larvae should exhibit low levels of population genetic differentiation. I predict that although life history may influence population genetic structure throughout much of the range of this

species, historical or ecological factors in the northern end of its range may have caused the large range disjunction found in this species and may be driving patterns of population genetic variation across this range disjunction.

Ch3: As part of a large collaborative effort among multiple Universities in Canada and the US, I develop a suite of microsatellite markers from a multi species pool of genomic DNA. I tested each marker for its species of origin, association with coding sequences in other non-asterinid species, and amplifications patterns that may influence patterns of polymorphism within and among species. My goal was to use these markers in comparative population genetic analyses; understanding the processes that drive patterns of polymorphism within and among species in these markers was of crucial importance.

Ch4: In this chapter I analyze population genetic data from 3-12 populations in 8 species of asterinid sea star using microsatellite markers. I describe the within- and between-population genetic variation for all 8 species.

Ch5: I explored the potential for outcrossing and brooding allometry in two live bearing asterinid species *Parvulastra vivipara* and *Parvulastra parvivipara.* I used microsatellite markers to examine broods for evidence of multiple paternity. I also examine brood characteristics such as offspring size and clutch size to explore the role of sibling conflict and maternal investment in this live bearing life history.

Ch6: In this chapter I evaluate the evolution of life history mode throughout the Asterinidae using a Bayesian ancestral character state reconstruction. This

chapter serves two purposes; first, it allowed me to determine the degree to which life history characters exhibited phylogenetic inertia (or were constrained by phylogeny) throughout this group. Secondly, I was able to examine the utility of ancestral character state reconstruction in comparative analyses using independent contrasts.

Ch7: In this chapter I use a Generalized Least Squares approach, accounting for phylogenetic relationship among species to conduct a comparative analysis of life history and population genetic structure in the Asterinidae. I evaluate the role of differing degrees of phylogenetic signal in my data. Finally, I assess the importance of accounting for phylogeny in the comparative analysis by comparing model fit in cases where phylogeny was accounted for and in those where it was left out.

**Figure 1.1: Phylogeny of all asterinid species with known life histories. Numbers described in the key indicate life history mode.**

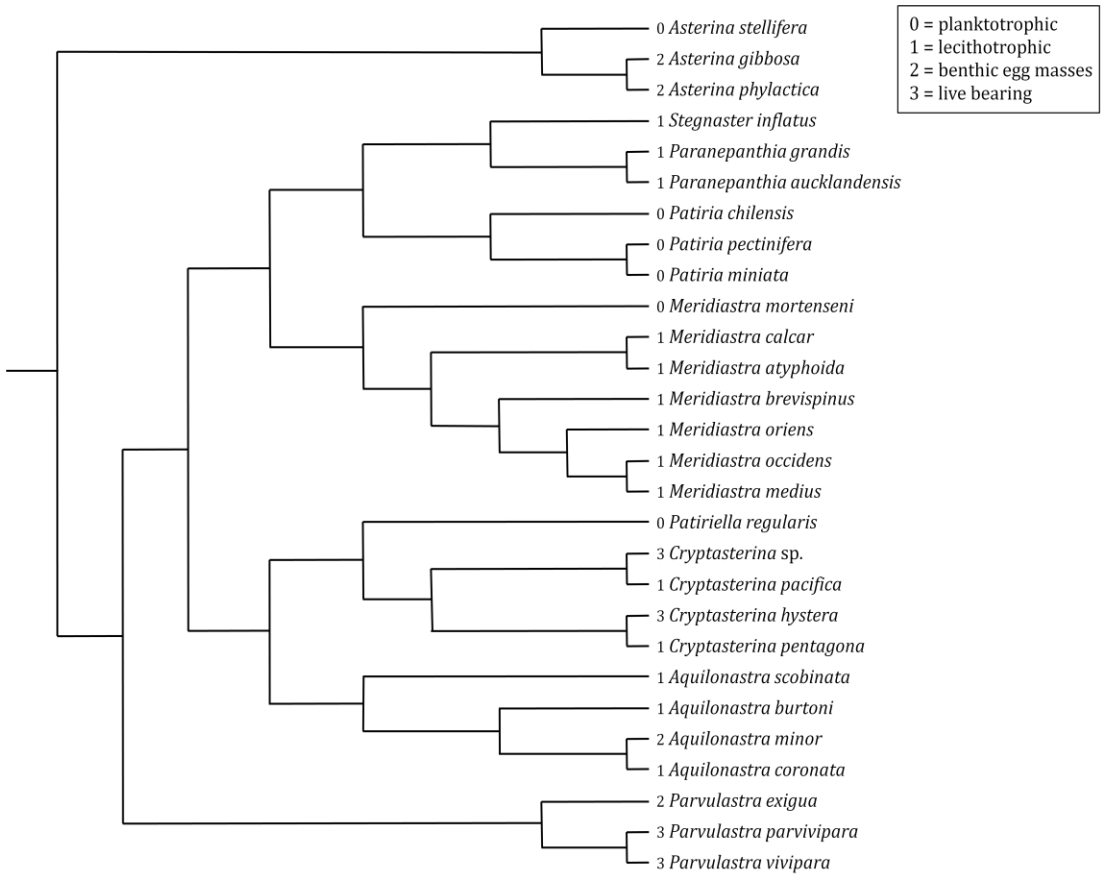
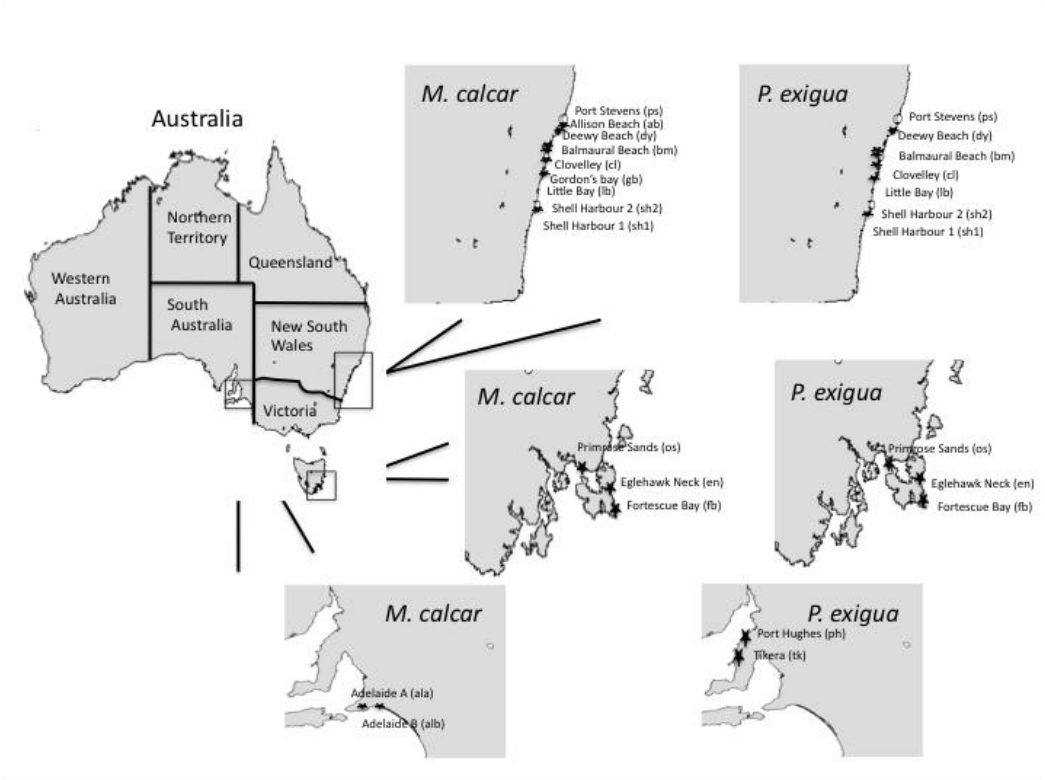
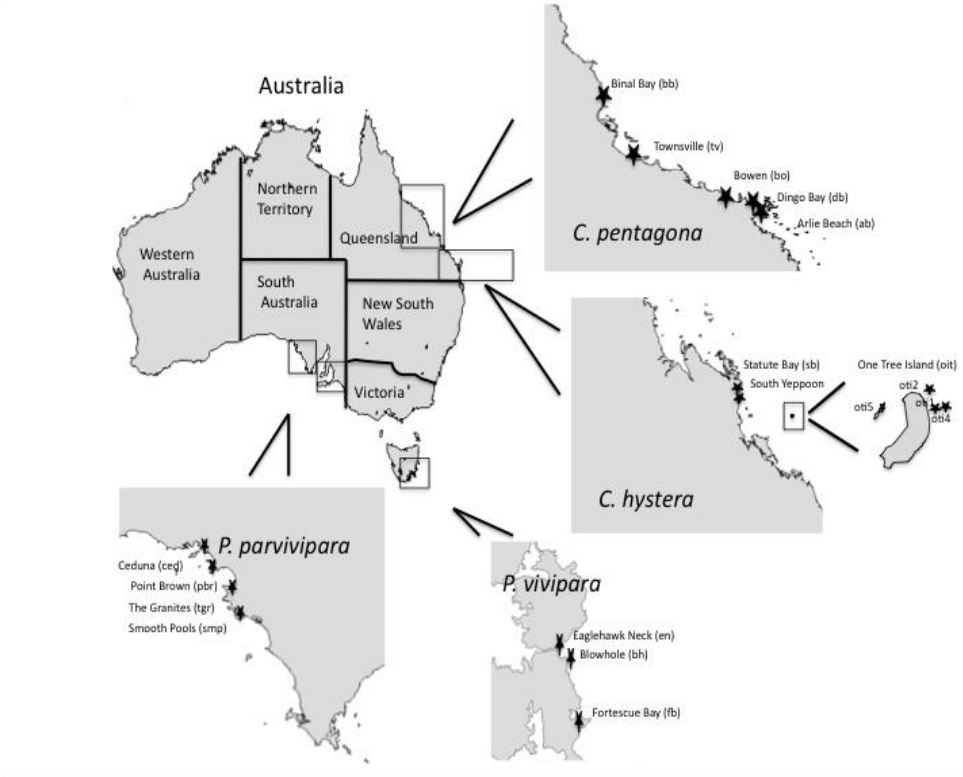
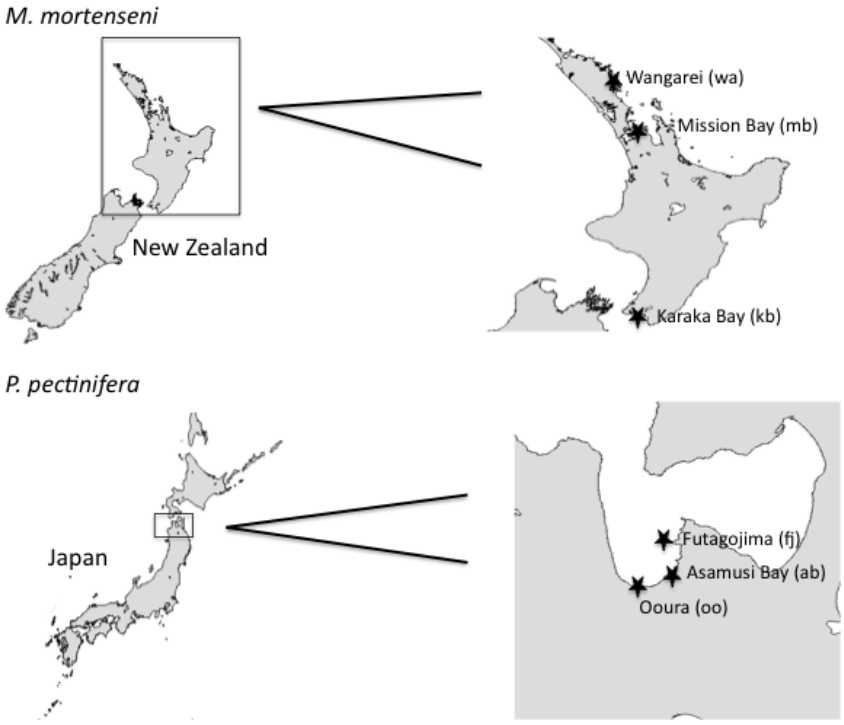


**Figure 1.2 Geographical sampling locations of *M. calcar*, *P. exigua, C.hystera, C. pentagona, P. parvivipara, P. vivipara, M. mortenseni* and *P. pectinifera.* Stars represent approximate sampling locations. References to life history strategies can be found in Figure 1.1.**




Australia
Northern Territory
Queensland
Western Australia
South Australia
New South Wales
Victoria
Binal Bay (bb)
Townsville (tv)
Bowen (bo)
Dingo Bay (db)
Arlie Beach (ab)
C. pentagona
Statute Bay (sb)
South Yeppoon
One Tree Island (oit)
oti2
oti5
oti4
C. hystera
P. parvivipara
Ceduna (ceq)
Point Brown (pbr)
The Granites (tgr)
Smooth Pools (smp)
P. vivipara
Eaglehawk Neck (en)
Blowhole (bh)
Fortescue Bay (fb)


M. mortenseni
New Zealand
Wangarei (wa)
Mission Bay (mb)
Karaka Bay (kb)
P. pectinifera
Japan
Futagojima (fj)
Asamusi Bay (ab)
Ooura (oo)

# Chapter 2 **POPULATION GENETIC ANALYSIS OF THE BAT STAR *PATIRIA MINATA* FROM THE NORTH EAST PACIFIC: DOES A PLANKTONIC LARVAL PHASE ALWAYS CONFER HIGH LEVELS OF CONNECTIVITY?**

This chapter was modified from Keever, C. C. J. Sunday, J. P. Puritz, J. A. Addison, R. J. Toonen, R. K. Grosberg, M. W. Hart. 2009. Discordant distribution of populations and genetic variation in a sea star with high dispersal potential. Evolution 63: 3214-3227, with permission from John Wiley and Sons.

## .Introduction

Factors that contribute to the patterns of genetic differentiation of marine species include intrinsic life history, historical and contemporary ecological or environmental factors such as oceanography, and species' behaviours and interactions (Avise 2001, Wares and Cunningham 2001, Knowles 2001, Grosberg and Cunningham 2001). For benthic marine invertebrates, the larval phase of the life history can be especially important in determining the extent of genetic differentiation through time and space (Scheltema 1971, Todd 1998, Hellberg 1996, Marko 2001). While larval dispersal potential is perhaps the most straightforward explanation for a species' genetic structure, it is not the only determinant. A species' population genetic structure can also be influenced by extrinsic factors such as historical vicariance, changing the extent and patterns of

dispersal, as well as the present-day extent of suitable habitat or ecological conditions for larvae and adults (Grantham et. al 2003, Marko 2004, Hickerson and Cunningham 2005).

Many marine invertebrate species posses a life history which includes a larval stage capable of swimming and feeding in the water column for days or weeks before settling (Strathmann 1985). Such species invest relatively less in nutrient reserves for each offspring, which increases fecundity by producing more individual offspring for any fixed amount of material allocated to egg production (Strathmann 1985, Raff 1992, Wray 1996, Villinski et.al 2002). Theory predicts that species with this type of life history will have a broad geographic distribution of individuals and alleles due to the requirement that these larvae feed and grow in the water column in order to complete early development (Emlet 1991, Emlet 1995, Bohonak 1999). The obligate broad dispersal of planktotrophic larvae is thought to prevent the deleterious effects of local inbreeding and extend the range across available patches of habitat separated by large distances (Knowlton and Jackson 2001). The genetic structure of such species should reflect low subdivision between populations and high genotype or haplotype diversity shared throughout the range.

Extrinsic barriers to dispersal can also be important. Many studies have found that some species have genetic structure that appears to be independent of their intrinsic dispersal abilities. First, Marko (2004) noted striking differences in population genetic structure among species in the same region despite having similar modes of benthic development. Likewise, Benzie (1999) found genetic

structure in species with dispersing planktonic larvae that have similar ranges to be severely out of accordance with predictions based on dispersal ability and oceanographic currents. Second, intrinsic dispersal abilities are not always maximized. Some studies (especially of marine fishes and crustaceans with relatively large muscular larvae) suggest that strong swimming ability plus complex sensory adaptations for orientation and habitat choice might promote philopatry in spite of prolonged planktonic development (Swearer et al. 1999). Third, historical biogeographic barriers and dispersal ability may interact. Broad comparative studies of single communities whose members share a common biogeographic history show that species differ in their susceptibility to the effects of changing dispersal barriers based on their intrinsic dispersal abilities (Teske 2007, Marko 2009).

Prominent among this last category are surveys of population genetic variation among fishes and invertebrates in the intertidal and shallow subtidal marine communities of the northeast Pacific (Arndt and Smith 1998; Burton 1998; Edmands 2001, Hellberg 2001, Marko 2004, Harley et al. 2006, Hickerson and Cunningham2006, Wilson 2006). Repeated Pleistocene glacial advance and retreat in the northern extent of this region undoubtedly formed extrinsic and dynamic dispersal barriers that seem to have affected species in different ways. Some members of this community show strong evidence of northern extirpation followed by recent recolonization from southern glacial refugia, while others show strong evidence of two (northern and southern) refugia, and vicariant isolation

between them. Evidence for both colonization patterns have been found among species with both high and low intrinsic dispersal abilities.

I use mitochondrial tRNA sequence data to investigate the distribution and genetic structure *Patiria miniata,* to determine the relative roles of life history, historical biogeography, and other ecological factors in its distribution and genetic structure. I find that although the broad geographic range of this species is consistent with the expected effects of a life history with a feeding planktonic larva, both contemporary and historical ecological factors may also be influencing the range of this species and its population genetic structure.

The sea star *Patiria miniata* (the bat star) exhibits an interesting geographic pattern in the eastern Pacific: it ranges from Baja California to Alaska but is conspicuously absent along the coast of Oregon and Washington (Figure 1) (Lambert 2000). The large gap in the distribution of *P. miniata* may be caused by some ecological factor, such as a lack of suitable habitat in the Oregon and Washington region, or by a combination of historical events and contemporary dispersal patterns. One possibility is that the Wisconsonian glacial maximum or another glacial period may be responsible for historical separation between populations north and south of the range gap, which has subsequently not been closed by resettlement.

*Patiria miniata* lives intertidally and subtidally and has external fertilization with a free-swimming larva that feeds in the plankton. Being an external fertilizing species with free-swimming larva, one would expect *P. miniata* to have a broad homogenous range (Bohonak 1999). However, several studies of

intertidal and subtidal marine invertebrate species along the west coast of north America show that life history may not be the best indicator of species response to historical process and that other aspects such as a adult ecology may have been more important during the last glacial cycle (Marko 2004).

The goal of this study is to analyze population genetic variation of the bat star *Patiria miniata* along the west coast of the US and Canada. I use mitochondrial transfer RNA sequence data to examine population genetic patterns of *Patiria miniata* throughout its range. I use this study as a baseline for my analysis of the relative role of a species' intrinsic dispersal ability in shaping population genetic structure. Mitochondrial markers are used because of the efficiency and quality of sequence data, and because many similar studies of population genetic structure of intertidal marine invertebrates along the coast of North America also use this type of marker to characterize population genetic structure (Edmands 2001, Marko 2004).

I investigate the unusual geographic distribution of the bat star *Patiria miniata* along the west coast of the US and Canada and first determine if geographic boundaries coincide with any genetic structure that I see. Secondly, I examine the relationship between the extent of genetic structure in this species and its relationship to intrinsic dispersal ability to determine if a species with planktonic larvae does indeed show highly homogenous population genetic structure throughout its range. Lastly, I speculate on a connection between the geographic range and genetic structure of *Patiria miniata* and a vicariance brought about by past climatic events. Many intertidal marine invertebrates from

the North East Pacific Ocean show genetic structure that indicates that historical climate processes may have caused vicariance of northern and southern populations and that these non-equilibrium forces are still at work in current population genetics of these species (Arndt and Smith 1998, Hellberg 2001, Edmands 2001, Sotka et al. 2004, Wares and Cunningham 2005). Furthermore, the glacial maximum 15-25 thousand years before present may contribute to the genetic structure of modern populations, as well as having caused the range disjunction that exists for this species (Hewitt 2000). I will examine two hypotheses that pertain to the current population genetic signal in *P. miniata,* and its relationship to past climactic events: (1) Recolonization and northward movement after the last glacial maximum (2) a refugial origin of the northern group of *P. miniata*.

## .Methods

I obtained tissue samples (tube feet) preserved in 70-95% ethanol from 423 individual sea stars collected from 14 locations in southeast Alaska, Haida Gwaii (the Queen Charlotte Islands), Vancouver Island, and California (Table 2.1).

I extracted DNA from tube feet using either a CTAB protocol (Grosberg et al. 1996) or a simple proteinase K digestion of a single tube foot in water (Addison and Hart 2005a). I used primers from Colgan et al. (2004) to amplify part of the mitochondrial genome that contains five transfer RNA genes and the 5' end of the cytochrome *c* oxidase subunit I (COI) gene (Hart et al. 1997).

Thermal cycling conditions were 90° (2:00), 55° (0:40), 72° (2:00) for 1 cycle; 90° (0:30), 55° (0:30), 72° (1:40) for 30 cycles; 90° (0:40), 55° (0:40), 72° (7:00) for one cycle. Amplicons were checked by agarose gel electrophoresis, purified by sodium acetate precipitation, and direct sequenced with the forward primer (Hart et al. 1997). Sequences were proof-read in 4Peaks v. 1.6 (A. Griekspoor and T. Groothuis, mekentosj.com), aligned in Clustal W (Thompson et al. 1997), and trimmed to standard length (369 bp; GenBank accession numbers EF165733-EF165790; EF165792-Ef165971; FJ939314-FJ939328).

**Quantitative Analysis**

For each population sample I calculated haplotype and nucleotide diversity using Arlequin v. 3.11 (Excoffier et al. 2005). I also used Arlequin to calculate Tajima's *D* and Fu and Li's *F* to test for departures from neutral variation in allelic diversity relative to the number of segregating sites (Tajima 1989) or number of alleles (Fu and Li 1993; Simonsen et al. 1995).

I constructed mismatch distributions for all populations in California, Vancouver Island and Alaska and the Queen Charlotte Islands independently. This analysis plots the frequency of pairwise nucleotide difference between individuals. A unimodal distribution of individuals with low pairwise difference indicates that populations are thought to have undergone recent demographic expansion but if the mode is at a higher number of pairwise differences then populations are suspected of having an expansion event sometime in the more distant past. If the distribution has multiple peaks, it is usually indicative of a

more complicated demographic history with multiple expansion periods throughout the past (Rodgers and Harpending 1992).

I examined spatial associations within haplotype networks based on the ancestor-descendant relationships among mtDNA using TCS (Clement et al. 2000). I calculated fixation indices (*F*-statistics) among all populations, between pairs of populations ($F_{ST}$), and among individuals within populations ($F_{IS}$), using Arlequin (Excoffier 2005). I used Mantel tests in Arlequin to characterize isolation by distance as the correlation between population differentiation (pairwise $F_{ST}$) and straight-line geographical distance. All *F*-statistics were computed by the method of Weir and Cockerham (1984), and *F*-values significantly different from zero were identified by comparison to results from 10000 permutations of the data (Raymond and Rousset 1995).

To test for regional subdivision of sequence diversity, I used analysis of molecular variance (AMOVA) in Arlequin. I used 10000 permutations of the data to identify measures of differentiation ($\Phi$) significantly different from zero. I partitioned this variance into differences between northern and southern population groups ($\Phi_{CT}$) and differences among populations within each group ($\Phi_{SC}$). I carried out two of these analyses: one based on population groups north (Alaska, Haida Gwaii, Vancouver Island) and south (all California sites) of the range disjunction; and a second based on the STRUCTURE results from microsatellite and nuclear sequence data (Keever et al. 2009) and from my TCS haplotype network, (Figure 2.1) which strongly suggested a phylogeographic

break between population groups north (Alaska, Haida Gwaii) and south (Vancouver Island, California) of Queen Charlotte Sound.

I conducted coalescent analyses of migration rate (*m*) and effective population size ($N_e$) for the mtDNA sequences using the Bayesian method in MIGRATE-N v. 2.4 (Beerli and Felsenstein 2001, Beerli 2006). Because my haplotype network analyses identified an unexpected and strong phylogeographic break at Queen Charlotte Sound in northern British Columbia, I was specifically interested in contrasting gene flow across Queen Charlotte Sound relative to gene flow across the range disjunction. I therefore organized my MIGRATE analyses to match my AMOVA analyses. I pooled all population samples from California, Vancouver Island, Haida Gwaii, and Alaska into four regional samples, and estimated the 16 corresponding parameter values (4 effective population sizes, 12 pairwise migration rates). For this number of parameters it was not possible to run MIGRATE using my full data set because the gene genealogies were prohibitively large. Consequently, I limited this analysis specifically to a total of 102 individuals from Alaska (n=17), Haida Gwaii (n=21), Vancouver Island (n=30), and California (n=34). The MCMC search was based on a chain of 50,000,000 (mtDNA) steps sampled every 40 or 100 steps for a total of 500,000 samples (with a burn-in of 50,000 samples). I used exponential prior distributions for migration rate (0, 1000) and effective population size (0, 0.01) because exponential priors more aggressively sample the parameter space. I assessed convergence by comparing results from multiple runs. I characterized effective population size as $\theta=4N_e\mu$, and expressed the

magnitude of gene flow estimated from these analyses as the number of migrants per generation $M=\theta m/\mu$.

## .Results

The mtDNA sequence alignment included 28 unique haplotypes that differed by up to 5 substitutions. There were 2-11 haplotypes per population, with haplotype diversities of 0.13-0.75 (Table 2.1). Nucleotide diversity was low ($\pi<0.004$) for these slow-evolving tRNA sequences but varied about four-fold among populations (Figure 2.2).

There was no association between latitude and mitochondrial diversity. After correction for 58 simultaneous tests of neutrality (*D* or *F*) across 13 populations, and 2 methods, *F* (from –5.56 to –22.04) from Louise Narrows, Tofino, and Bodega Bay were significantly different from zero (neutral expectation). No values of *D* were significantly different from zero. The bias toward a few significant *F* (rather than *D*) values could reflect the information from invariant sites incorporated into the *F* statistic (346/369 bp). The result suggests some isolated cases of non-neutral excess of low frequency mutations in individuals relative to segregating sites or number of alleles consistent with recent population expansion.

### Population Structure

The geographical distribution of haplotypes exhibited two general groupings: one group of samples occurred mainly in California and Vancouver

Island, and the other was mainly restricted to Haida Gwaii and Alaska (Fig 2.1 and 2.2 for different representations). Most individuals (88%) had one of three haplotypes that differed from each other by one or two substitutions; four other shared haplotypes occurred in 2-5 individuals. Of the three most common haplotypes, only one (n=42) appeared in all four geographical regions from Alaska to California. The single most common haplotype (n=120) was entirely absent from our large sample of sea stars from Alaska and was rare in Haida Gwaii. The second most common haplotype (n=59) was restricted to these two northern regions, as were two other shared haplotypes. Altogether, 70 of 96 DNA sequences from Alaska and Haida Gwaii samples were unique to those two regions north of Queen Charlotte Sound. South of Queen Charlotte Sound, the Vancouver Island and California populations shared two of the three most common haplotypes as well as two of the most rare haplotypes (Figure 2.1 and 2.2 for different representations).

All populations from Vancouver Island and California showed unimodal mismatch distributions indicative of population expansion (Figure 2.3). Three out of five California populations had mismatch distributions with modes at one, which indicates that these populations have had expansions sometime further in the past than other California populations or Vancouver Island populations, which all had a mode at 0 differences. Interestingly, the two California Populations with modes at 0 represent the edges of the sampling range in this state (i.e. Fort Bragg and Santa Barbara). The test for Harpending's Raggedness Index revealed no significant deviation from recent population expansion (at any time in

the past) for any population sampled (r = 0.10 – 0.55, P = 0.17 – 0.92). That said some populations from Alaska and Haida Gwaii (VC, DI TU) have bimodal mismatch distributions, suggesting that populations may not have experienced one but potentially multiple growth events throughout the past (e.g. Tanu: 0 and 2).

**Population Differentiation**

Global measures of deviation from equilibrium allele frequencies differed significantly from zero and suggested a considerable history of genetic drift within populations and changes in allele frequencies among some populations (Table 2.3). Population pairwise $F_{ST}$ values were typically lower in comparisons among populations north or south of Queen Charlotte Sound, and considerably higher in comparisons between populations separated by Queen Charlotte Sound (Table 2.2). Mantel tests identified weak signals of isolation-by-distance ($r$=0.32, $P$ =0.022), but this correlation was not significantly different from zero after Bonferroni correction for four simultaneous tests.

In an AMOVA that grouped populations into regions north and south of the range disjunction, differentiation between groups was small ($\Phi_{CT} \leq 0.07$), was not significantly different from zero, and accounted for 7.5% of molecular variance. A large proportion of the molecular variance (21%) was due to differences among populations within these two groups, in particular to the many large pairwise differences between populations from Vancouver Island and those from Alaska and Haida Gwaii (Table 2.3). In contrast, when I grouped populations north and

south of Queen Charlotte Sound (the population grouping found in the STRUCTURE analysis in Keever et al 2009), I found considerably greater differentiation between regional groups (as high as $\Phi_{CT}$=0.33; Table 2.3).

**Coalescent Analysis**

The analyses above based on genetic distances consistently revealed one major phylogeographic break in *Patiria miniata*. I used the coalescent analyses to explore two aspects of this population genetic structure: (1) demographic differences among populations manifested as variation in effective population size; and (2) asymmetries in gene flow that might underlie patterns of allele frequency similarities between populations.

Analysis of gene flow from the mtDNA data did not identify any significant differences in effective population size or of migration rates among these four regions. In general, the MCMC search converged on coalescent patterns that emphasized widespread ancestral polymorphisms within populations to account for allele or haplotype sharing rather than high and variable rates of migration between populations (Figure 2.5). Effective population size was lower in Alaska relative to the other three regions however all four estimates had largely overlapping confidence intervals (see table in Figure 2.4). Similarly, migration rates were lower between some sets of populations on either side of Queen Charlotte Sound but the magnitude of these differences were not significantly different from each other using a 95% confidence interval (table in Figure 2.4).

Although mtDNA estimates of migration rate and effective population size for P. miniata populations proved inconclusive, estimates from nuclear intron data as well as data from anonymous nuclear loci show significantly higher migration rates among populations on either side of Queen Charlotte Sound, and significantly higher effective population sizes for some California and Vancouver Island populations, relative Alaska to and Haida Gwaii populations (Keever et al. 2009, McGovern et al*. in press*). These data are not explicitly included in the information of this thesis, but are part of a larger collaboration examining the relative contribution of life history to population genetic structure and also examining the utility of different classes of molecular markers in phylogeographic studies.

## .Discussion

Two consistent and strong population genetic patterns emerged from my analysis of genetic structure in the bat star. First, there was a strong and highly significant pattern of population genetic differentiation between *Patiria miniata* populations north and south of Queen Charlotte Sound, including populations from southern Haida Gwaii and northern Vancouver Island separated by just a few hundred kilometres. Second, there was no evidence for genetic differentiation across the majority of the bat star geographic range from Vancouver Island to southern California that includes the broad distributional gap in Washington, Oregon, and northern California.

In the absence of genetic information, the coincidence of the *P. miniata* geographic range disjunction and the southern extent of the last North American glaciation on the Pacific coast might imply that bat star populations gradually expanded out of northern and southern glacial refuges, leaving descendants that established the extant populations north and south (respectively) of the range disjunction. However, the discordance between the location of the phylogeographic break, the geographic range disjunction and the genetic affinity between Vancouver Island and California populations causes me to reject this hypothesis.

Although information on gene flow was inconclusive my results suggest that one of three ongoing scenarios is driving the patterns of population genetic differentiation. First, there could be high gene flow across the massive range disjunction of *P. miniata*. High dispersal potential of planktonic larval stage makes this explanation probable, although the extent of migration could not be calculated with any certainty using my mitochondrial sequences. Second, there could have been one recent colonization event of Vancouver Island populations from California. The mismatch distributions show a strong peak at 0 pairwise differences between individuals in the Vancouver Island populations which suggests that these populations have gone through a recent population expansion (Figure 2.3). A third scenario (which would be difficult to reject on the basis of genetic data alone) is recent local extirpation of bat star populations in Washington, Oregon, and northern California that fragmented a formerly more continuous species range.

The origin and maintenance of the phylogeographic break at Queen Charlotte Sound may be more likely associated with geological and oceanographic dispersal barriers. A coalescent estimate of population divergence time between bat stars from Alaska and Vancouver Island using mtDNA and six anonymous nuclear DNA sequence markers (T. McGovern, C. Keever, M. Hart, C. Saski, and P. Marko, unpublished data) is ~280,000 years ago. This divergence time is consistent with long-term population persistence and potentially isolation due to changes in climate, sea level, and oceanographic circulation associated with the last glacial episode (or with other ecological or geological processes operating on the same time scale but not associated with glacial cycles). Similarly, mismatch distributions, which are often used to speculate on demographic patterns (Mousset et al. 2004), show a two-fold higher average pairwise difference among individuals in populations north of Queen Charlotte Sound and single-fold higher average pairwise difference among individuals in populations in California relative to Vancouver Island Populations. These results suggest that there were possibly older population expansion periods north of Queen Charlotte Sound and in California relative to Vancouver Island populations and in fact, a study by Marko et al. (2009) shows that population expansion of *P. miniata* based on mismatch analyses predates the last glacial maximum (about 15,000 years before present) occurring as much as 290-330 thousand years before present.

The phylogeographic break I observed coincides geographically with a west-to-east surface current (the North Pacific Current) that diverges to form the

northward Alaska and southward California current systems (Cummins and Freeland 2007). High-resolution models of density- and wind-driven circulation in the northeast Pacific (Foreman et al. 2008) suggest that exchange of *P. miniata* larvae across Queen Charlotte Sound may be limited because of the North Pacific Current. This physical current structure could contribute to the maintenance of a phylogeographic break established by Pleistocene climate change in spite of the prolonged 6- to 10-week period of planktonic larval growth and development in bat stars (Strathmann 1987; Rumrill 1989; Basch 1996).

**Comparisons to Phylogeographic Studies of Close Neighbours**

Bat stars share a similar phylogeographic break with a taxonomically heterogeneous group of snails, fishes, and sea cucumbers (see Arndt and Smith, Marko 2004, Hickerson and Cunningham 2005). These species all lack a long-lived planktonic larval stage, and so my study is the first in this well-known zoogeographic transition zone to document strong population genetic differentiation in a species with high intrinsic dispersal potential. My results contrast with those for the abundant keystone predator *Pisaster ochraceus*, which also occurs in this region and, like the bat star, has planktotrophic development. Harley et al. (2006) found no significant mtDNA differentiation in P. ochraceus at Queen Charlotte Sound (or at any other well-known phylogeographic breaks such as Point Conception in California; Dawson 2001). Although *Pisaster ochraceus* and *Patiria miniata* have broadly overlapping geographical distributions and similar modes of larval dispersal, they occur in

different habitats and on different substrates (Lambert 2000). If the phylogeographic break between bat star populations was initiated by early Pleistocene glaciations and maintained by the North Pacific Current (as suggested above), these two species may also differ in their response to Pleistocene climate change (and population history) or in their response to present-day ocean currents and the tendency to cross Queen Charlotte Sound during larval development. The nature of such differences is unknown.

More recently, Marko et al. (2009) found little evidence for a relationship between ecological niche or larval dispersal mode (planktonic or benthic) and response to historical isolation across Queen Charlotte Sound. In this study they examined 14 N.E. Pacific species and found that while some species showed evidence of persistence in northern populations throughout the last glacial maximum, several others showed recent extirpation and recolonization in accordance with glacial recession. Among the 5 included planktonic species all but one (*Evasterias troschelii)* showed evidence of long-term persistence through the Pleistocene. Although the correlations between historical isolation and life history in Marko (2009) are somewhat inconclusive, with greater species sampling a pattern that corresponds to a common intrinsic property of organisms may emerge. Alternatively, it may be that community dynamics (i.e. species' interactions with each other and their environment) play a larger role than life history in the persistence or extirpation of species over time (Helmuth et al. 2006, Harley and Paine 2009). If this is the case then as these communities change through time the responses of the species within them to climatic fluctuations

should change as well, weakening the chance of finding any observable pattern in species' response to climate change.

**Relevance to Comparisons Among Asterinids**

The widespread distribution and the lack of genetic structure across a large geographical range disjunction (in Oregon and Washington) in *P. miniata*, coupled with findings from other Asterinid species with benthic development which appear to have high levels of population genetic differentiation and restricted gene flow, suggests that changes in life history may in some cases be important determinants of population genetic structure in asterinids (Hunt 1993; Matsuoka and Asano 2003; Waters et al. 2004; Baus et al. 2005; Colgan et al. 2005; Sherman et al. 2008). However, the discovery of unexpected and strong phylogeographic breaks in *P. miniata* and another asterinid (from New Zealand; Waters and Roy 2004) with planktotrophic development, and fewer phylogeographic breaks in benthic developers (Ayre et al. 2009) suggests that extrinsic physical or geological barriers and historical processes might also have significant effects on population genetic variation in asterinids.

All factors must be taken into account when conducting comparative analyses, and this thesis I will proceed with this in mind. I examine population genetic structure in asterinids as it relates to both life history and important extrinsic barriers to dispersal including previously investigated biogeographic and phylogeographic breaks and prevailing ocean currents. Only after considering all likely intrinsically and extrinsically specific scenarios of population genetic

variation will I more closely examine the relationship between life history and genetic differentiation in the context of phylogeny.

## .General Conclusion

My study, examining the population genetic structure and phylogeographic patterns of *P. miniata* across its range is extremely important in that it informs predictions for comparisons among asterinids with similar modes of development evolved in parallel. While I expect changes in life history found among the asterinids to be an important predictor of genetic structure, I am also aware of the important influence of historical demography and contemporary ecological factors in generating population genetic differentiation.

Given my marker choice in this chapter, it was difficult to differentiate between gene flow, genetic drift, and historical processes in driving patterns of population genetic differentiation. Subsequent comparative analyses based on nuclear sequence data have been able to resolve some of these issues and to infer migration rates with certainty in this species (Marko et al. 2009, Keever et al. 2009).

Recent studies by Hart and Marko (2010) and McGovern et al. (2010) compared patterns of population genetic variation in the bat star *P. miniata* to a sympatric gastropod species *Nucella lamellosa* using multilocus coalescent analyses. They found that multilocus IMa (Hey and Nielsen 2007) estimates of divergence time in *P. miniata* are several orders of magnitude older across Queen Charlotte Sound than that of *Nucella lamellosa*, a species which shows

no phylogeographic structure across this same region and that lacks planktonic larval dispersal. Similarly, although *P. miniata* shows a significant phylogeographic break in this region relative to *N. lamellosa* estimates of gene flow from IMa analyses are non-zero in the bat star and do not significantly differ from zero in the snail species. These results suggest that the differences in phylogeographic structure between the two species may be a result of their differing responses to historical vicariant process, both at the end of the last glacial maximum (~10,000 years) and the more distant past, rather than differences in the species' contemporary dispersal potential which dictate their migratory abilities. Similarly, Hart and Marko (2010) and McGovern et al. (2010) found that migration rates for *P. miniata* were non-zero and concordant across the entire range of this species, suggesting that gene flow via migration of planktonic larvae may be relatively similar throughout the species range, and that phylogeographic signatures in this species are the result of barriers to gene flow in the distant past.

The results of my analysis of population genetic differentiation are consistent across microsatellites and nuclear intron sequence data. The remainder of the data collection for this thesis was conducted using microsatellites (see Ch 3). This data will be part of a large collaborative effort to examine the correlation between life history mode and population genetic structure in the Asterinidae. The high variability of microsatellite markers can show fine scale genetic differences between populations that may not otherwise be detected using mtDNA. However, the unknown mutational properties of these

markers make them difficult to use in coalescent-based analyses (see Chapter 3).

**Table 2.1 *Patiria miniata* sampling locations, including latitude and longitude, sample size (n) and mtDNA estimates of haplotype and nucleotide diversity.**

| Population/Region | Lattitude | Longitude | n | haplotype diversity | nucleotide diversity |
|---|---|---|---|---|---|
| **Alaska** | | | | | |
| View Cove | 55◦ 05′ 01′′ N | 133◦ 01′ 05′′ W | 36 | 0.52 | 0.0029 |
| Dunbar Inlet | 55◦ 04′ 09′′ N | 132◦ 50′ 47′′ W | 27 | 0.36 | 0.0019 |
| **Haida Gwaii** | | | | | |
| Rennel Sound | 53◦ 24′ 24′′ N | 132◦ 31′ 39′′ W | 12 | 0.75 | 0.0031 |
| Moresby Camp | 53◦ 03′ 07′′ N | 132◦ 01′ 32′′ W | 13 | 0.68 | 0.0036 |
| Tanu | 52◦ 45′ 55′′ N | 131◦ 36′ 55′′ W | 11 | 0.6 | 0.003 |
| **Vancouer Island** | | | | | |
| Winter Harbour | 50◦ 31′ 16′′ N | 128◦ 01′ 31′′ W | 18 | 0.4 | 0.0017 |
| Tofino | 49◦ 08′ 38′′ N | 125◦ 53′ 30′′ W | 31 | 0.3 | 0.0008 |
| Bamfield | 48◦ 49′ 43′′ N | 125◦ 08′ 05′′ W | 32 | 0.5 | 0.0018 |
| **California** | | | | | |
| Fort Bragg | 39◦ 24′ 32′′ N | 123◦ 48′ 22′′ W | 15 | 0.13 | 0.0003 |
| Bodega Bay | 38◦ 19′ 09′′ N | 123◦ 02′ 59′′ W | 23 | 0.62 | 0.002 |
| Monterey | 36◦ 37′ 15′′ N | 121◦ 54′ 16′′ W | 5 | 0.7 | 0.0027 |
| Carmel | 36◦ 32′ 39′′ N | 121◦ 54′ 12′′ W | 10 | 0.53 | 0.0016 |
| Santa Barbara | 34◦ 27′ 02′′ N | 119◦ 42′ 05′′ W | 20 | 0.36 | 0.0012 |

**Table 2.2 Analysis of molecular variance (AMOVA) comparing genetic variance at different scales. (A) Analysis performed on all populations. (B) Analysis performed on populations placed into two groups, 1) all populations north of Queen Charlotte Sound and 2)all populations south of Queen Charlotte sound, based on the finding of a range disjunction in this region (Keever et al. 2009). (C) Analysis performed on populations grouped relative to the range disjunction (i.e., no bat stars occur along Washington and Oregon coasts), comparing southern (California) populations to all northern populations.**

| | | **No Geographic Grouping** | |
|---|---|---|---|
| A. | %variance | Fixation index | p-value |
| Among populations | 25.5 | F ST =0.25 | <0.001 |
| Within populations | 74.4 | | |
| | | **Range Disjunction** | |
| B. | %variance | Fixation index | p-value |
| Among groups | 7.5 | CT=0.07 | 0.137 |
| Among populations \| groups | 21.1 | ST=0.28 | <0.001 |
| Within populations | 71.3 | SC=0.22 | <0.001 |
| | | **Phylogeographic Break** | |
| C. | %variance | Fixation index | p-value |
| Among groups | 33.9 | CT=0.33 | <0.001 |
| Among populations \| groups | 3.6 | ST=0.37 | <0.001 |
| Within populations | 62.4 | SC=0.05 | 0.005 |

**Table 2.3 Mitochondrial DNA pairwise Fst estimates based on the method of Weir and Cockerham. Details of sampled populations can be found in Table 2.1. Population abbreviations are as follows: View Cover Alaska (VC), Dunbar Inlet (DI), Louise Narrows (LN), Moresby Camp (MC), Rennelle Sound (RS), Tanu (TA), Winter Harbour (WH), Tofino (TO), Bamfield (BA), Fort Bragg (FB), Bodega Bay (BOD), Hopkins Marine Station (HOP), and Carmel (CAR), Santa Barbara (SB).**

mtDNA

| | VC | DI | LN | MC | RS | TA | WH | TO | BA | FB | BOD | HOP | CAR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DI | -0.0032 | | | | | | | | | | | | |
| LN | | | | | | | | | | | | | |
| MC | 0.1172 | 0.2633 | | | | | | | | | | | |
| RS | 0.1737 | 0.3056 | | -0.0728 | | | | | | | | | |
| TA | 0.0364 | 0.0094 | | 0.2084 | 0.2088 | | | | | | | | |
| WH | 0.3545 | 0.4788 | | 0.2481 | 0.1836 | 0.3998 | | | | | | | |
| TO | 0.4155 | 0.5535 | | 0.3582 | 0.2805 | 0.5102 | -0.0028 | | | | | | |
| BA | 0.3441 | 0.4777 | | 0.1716 | 0.1188 | 0.4318 | 0.0312 | 0.0571 | | | | | |
| FB | 0.3718 | 0.5399 | | 0.2971 | 0.2130 | 0.4908 | -0.0116 | -0.0299 | 0.0111 | | | | |
| BOD | 0.3271 | 0.4468 | | 0.1831 | 0.1293 | 0.3750 | -0.0084 | 0.0061 | -0.0009 | -0.0258 | | | |
| HOP | 0.2559 | 0.4372 | | -0.0240 | -0.0440 | 0.3340 | 0.1189 | 0.2480 | 0.0130 | 0.2482 | 0.0120 | | |
| CAR | 0.3168 | 0.4629 | | 0.1778 | 0.1147 | 0.3660 | -0.0262 | 0.0081 | -0.0213 | -0.0167 | -0.0271 | 0.0543 | |
| SB | 0.3429 | 0.4899 | | 0.2043 | 0.1422 | 0.4323 | 0.0062 | 0.0192 | -0.0137 | -0.0222 | -0.0203 | 0.0625 | -0.0216 |

**Figure 2.1 MtDNA haplotype network for *P. miniata.* Each circle represents a different haplotype. Each colour represents a geographic region. The area of each circle (and the segments of each pie diagram) is proportional to the haplotype frequency. Small circles with solid colours represent singletons within a particular region. Lines between symbols indicate single substitutions and the small open symbols indicate one missing haplotype where one sequence differed by two substitutions from the most similar sequence. Black rings on the map show approximate location of samples. Grey and white lines represent approximate range disjunction. The arrows indicate the approximate location of genetic divergence and also represent the approximate location of divergence of the North Pacific Current (NPC) into the Alaska and California Currents.**

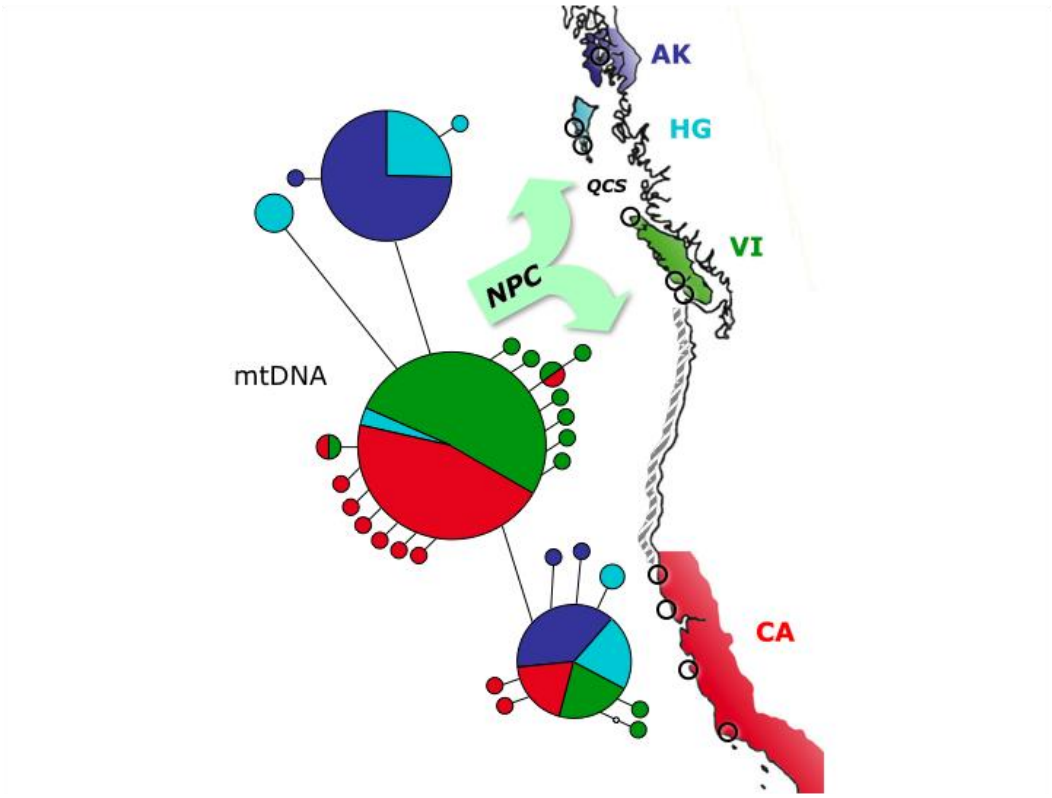


**Figure 2.2 Haplotype frequency distributions by population and their corresponding diversity indices. Y-axes are haplotype frequencies that range from 0 to 1 for each region (California, Vancouver Island, Haida Gwaii and Alaska). X-axes represent each haplotype in my dataset. Shown next to each histogram of haplotype frequencies are estimates of gene (haplotype) and nucleotide diversities for each region.**

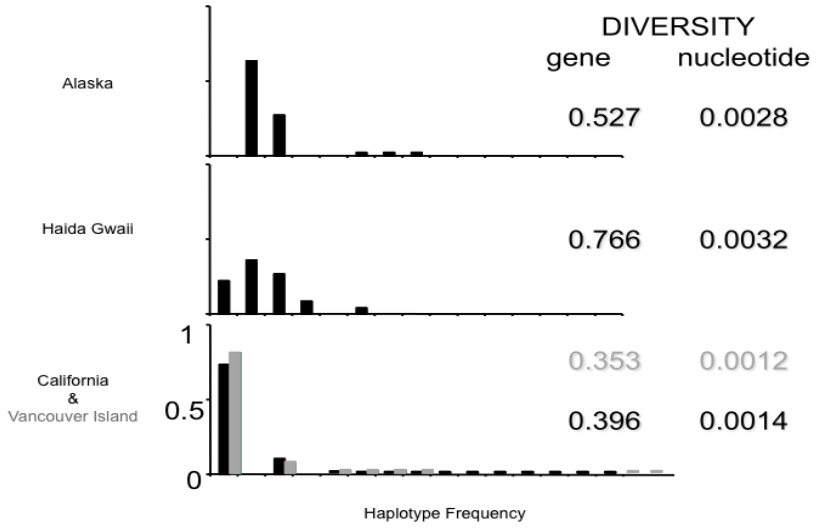


**Figure 2.3 Pairwise mismatch distributions for each *P. miniata* population. The X-axis represents the number of pairwise differences among sequences and the Y-axis represents frequency of pairwise distances among sequences within a population. One population can be found per graph and regions are separated by horizontal dotted lines.**

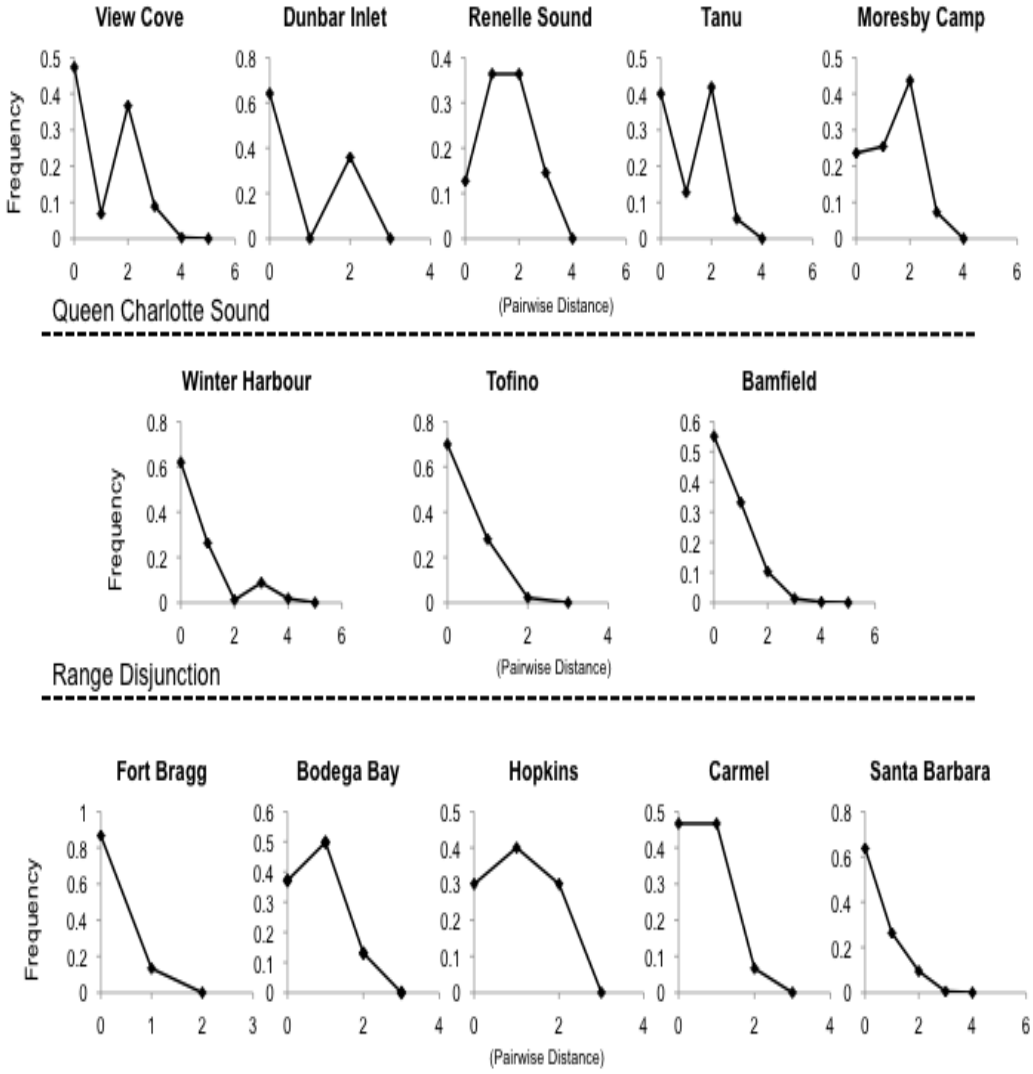
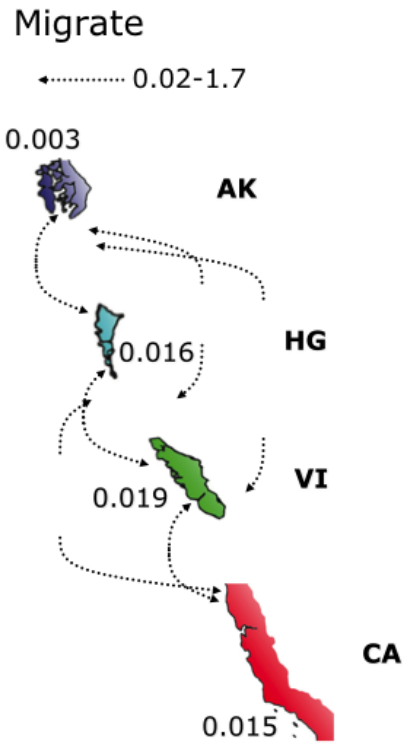


**Figure 2.4 Asymmetrical gene flow (as migrants per generation $M = \theta m/\mu$) and effective population size ($\theta$) estimated from mtDNA. Populations are grouped into four regional samples. Arrows show gene flow from one region to another. The dotted line represents 0.02 to 1.7 migrants per generation. Numbers show effective population size for each region. Arrows indicate the direction of gene flow. Migration estimates and their 95% confidence intervals are shown in the inset table.**




| Theta | | ML | 95% | |
|---|---|---|---|---|
| AK | | **0.0031** | 0.0011 | 0.0152 |
| HG | | **0.0163** | 0.0023 | 0.0505 |
| VI | | **0.0189** | 0.0029 | 0.0649 |
| CA | | **0.0148** | 0.0029 | 0.0448 |

| *M* | | ML | 95% | |
|---|---|---|---|---|
| AK → | HG | **1.7586** | 0.041 | 9.0388 |
| | VI | **0.2364** | 0.0472 | 7.7062 |
| | CA | **0.2601** | 0.0371 | 5.3883 |
| HG → | AK | **0.1324** | 0.0078 | 1.1921 |
| | VI | **0.1414** | 0.0472 | 5.0421 |
| | CA | **0.1116** | 0.0372 | 3.7563 |
| VI → | AK | **0.0235** | 0.0078 | 0.8523 |
| | HG | **0.204** | 0.0408 | 5.4269 |
| | CA | **0.1856** | 0.0371 | 5.5306 |
| CA → | AK | **0.0234** | 0.0078 | 0.7879 |
| | HG | **0.2035** | 0.0406 | 5.4132 |
| | VI | **0.236** | 0.0472 | 9.3016 |

# Chapter 3 **DISCOVERY AND CROSS-AMPLIFICATION OF MICROSATELLITE POLYMORPHISMS IN ASTERINID SEA STARS**

This chapter was modified from Keever, Carson C., Jenifer Sunday, Charlene Wood, Maria Byrne, Michael W. Hart. 2008. Discovery and Cross-Amplification of Microsatellite Polymorphisms in Asterinid Sea Stars. Biological Bulletin. 215: 164-172. with permission from the publisher, Marine Biological Laboratory, Woods Hole MA.

## .General Introduction

In this chapter I describe microsatellite marker development from mixed species pooled genomic DNA, used for comparative population genetic analysis and mating system studies of asterinid sea stars. High levels of polymorphism, bi-parental inheritance, and ability to genotype many loci at low cost make microsatellites attractive for a wide variety of population genetic analyses (Selkoe and Toonen 2006).

A useful property of many microsatellite markers is their cross-amplification in both focal species (in which the microsatellite was cloned) and in non-focal species, for which the markers cross-amplify (Vowles and Amos 2006, Amos et al. 2003, Hutter et al. 1998, Primmer et al. 1995, Amos et al. 1996). Many studies of microsatellite cross amplification have found an ascertainment

bias toward larger fragment sizes in the focal species relative to alleles at the same locus in non-focal species. The ascertainment bias is caused by the overrepresentation of clones with long microsatellite repeats at the selection or enrichment step of microsatellite discovery (Ellegren et al. 1995). For any unbiased distribution of allele sizes among species, the selective discovery of only loci with long alleles in the focal species will tend to produce a higher frequency of shorter allele sizes among other (non-focal) species. Furthermore, because mutation rates increase with repeat length (due to slippage during DNA replication), allelic diversity is also expected to be greater in focal species than at the same loci in a non-focal species (Ellegren et al. 1995).

The potential influence of ascertainment bias on allele size and polymorphism prompted me to investigate cross-amplification patterns among species and loci. Ascertainment bias has the potential to confound population genetic analyses by biasing length and thus levels of polymorphism toward higher values in focal species than in non-focal species. Because I use allelic diversity as a population genetic parameter in my comparative study, it was important to determine the potential effect of ascertainment bias (e.g., in species for which most available microsatellite markers Ire originally cloned from another species) relative to the effect of life history differences on levels of allelic diversity.

Here I compare cross amplification patterns, levels of variation and average allele size in focal and non-focal species. I also examine patterns of cross amplification and levels of variation in some loci that were found to be

associated with coding sequences in BLAST searches. Ultimately, I find that patterns of polymorphism are decoupled from patterns of allele size, in comparisons of focal and non-focal species. I find a strong relationship between levels of variation (measured as allelic richness) and patterns of cross amplification, but I find no association between allele size and patterns of cross amplification. This decoupling suggests that processes (such as the evolution of life history differences) other than the effect of ascertainment bias on allele size may be driving allelic associations and patterns of diversity.

## .Abstract

Variation in tandem repeats of two- to six-base nucleotide motifs (microsatellites) can be used to obtain inexpensive and highly informative multi-locus population genetic data. I develop and test a large set of cross-amplifiable sea star (Asterinidae) microsatellite markers from an eight-species mixed genomic DNA pool. I describe cloned sequences, primers, and PCR conditions, and characterize population-level variation for some species and markers. A few clones containing microsatellites show considerable similarity to sequences (including genes of known function) in other sea stars and in sea urchins (from the *Strongylocentrotus purpuratus* complete genome). The pooled genomic DNA method was an efficient way to sample microsatellites from many species: I cloned 2-10 microsatellites from each of eight species, and most could be cross-amplified in 1-7 other species. At 20 loci in nine species, I found 1-26 alleles per microsatellite, with a broad range of inbreeding coefficients. Measures of

polymorphism were not correlated with the extent of cross-amplification or the genetic distance from the focal species. Excluding one locus the average allele size among loci was not clearly correlated with extent of cross amplification, nor the genetic distance from the focal species, suggesting factors other than ascertainment bias may be working to shape the microsatellite amplification patterns and levels of polymorphism.

## .Introduction

The highly variable dispersal potential and mating systems of asterinid sea stars (e.g., Byrne, 1995, 2005, 2006; Byrne and Cerra, 1995; Byrne *et al.,* 2003; Hart et al., 2006) combined with specific conservation concerns (Emson and Crump, 1984; Law and Kelly, 2004; Tasmania Threatened Species Protection Act 1995) makes these species particularly interesting for population genetic analysis. An early study used multiple allozyme loci (Hunt, 1993) to compare two abundant intertidal Australian asterinid species with different dispersal biology over a small sympatric portion of their extensive geographic ranges. More recent population genetic studies used anonymous dominant nuclear markers (AFLPs; Baus *et al.,* 2005) or a single mtDNA locus (Colgan *et al.,* 2005). Other mtDNA surveys that emphasized phylogeographic hypotheses have been limited by cryptic species diversity (Waters and Roy, 2004a; Hart *et al.,* 2006) and small population samples (Waters and Roy, 2004b; Waters *et al.,* 2004a).

Analysis of microsatellite allele size variation offers several advantages over other classes of genetic markers (Selkoe and Toonen, 2006): co-

dominance, high polymorphism, low cost per sample and locus, large numbers of variable loci with potential broad coverage of the genome, and analysis of preserved or very small tissue samples (even single embryos) (Hellberg 2009). Microsatellites are known from two sea star species, the crown-of-thorns *Acanthaster planci,* and more recently for *Asterina gibbosa* (Acevedo et al. 2009; Yasuda *et al*., 2006), but appear to be rare in the genomes of some other sea stars (Baus *et al.,* 2005; Harper and Hart, 2005). Here I describe the development and application of a large new suite of microsatellite markers for comparative analysis of asterinid population genetic variation. Genotyping results show among-locus variation in polymorphism, inbreeding coefficients, and range of cross-amplification. I note some interesting correlations among these three variables, and the implications for use of different marker combinations in analyses of population structure.

## Materials and methods

### Microsatellite Identification from Pooled Genomic DNA

I used standard proteinase K digestion and chloroform extraction methods to obtain genomic DNA from gonads (of large-bodied species) or whole rays (in some small-bodied species). I sampled two to five individuals from each of eight asterinid species in five genera collected from shallow coastal habitats of southern and eastern Australia and western North America: *Cryptasterina hystera* (Dartnall et al. 2003) and *C. pentagona* (Müller and Troschel, 1842) from central and northern Queensland, respectively; *Meridiastra calcar* (Lamarck,

1816) and *M. oriens* (O'Loughlin 2002) from New South Wales; *Parvulastra exigua* (Lamarck, 1816) (New South Wales) and *P. parvivipara* Keough and Dartnall, 1978 (South Australia); *Patiria miniata* (Brandt, 1835) (British Columbia, Canada); *Patiriella regularis* (Verrill, 1867) (Tasmania). I chose these species in order to increase the likelihood that I would sample microsatellites from different clades, modes of reproduction, and biogeographic regions.

Genetic Identification Services (GIS, Chatsworth, CA; www.genetic-id-services.com) pooled similar amounts of high molecular weight genomic DNA from each species above (one or two individuals per species; 10 individuals in total). This bulk genomic DNA was partially digested and then size-selected for fragments about 300-700 bp in length. These fragments were cloned into plasmid libraries enriched for CA, ATG, CAG, and TAGA microsatellite motifs using a method (Jones *et al.,* 2002) that eliminates the need for probing (for detailed methods and recent examples see Tarvin, 2006; Carlon and Lippé, 2007; Hull *et al.,* 2007). I deliberately sequenced and PCR-tested more ATG and CAG clones and fewer CA or TAGA clones as a compromise between the expected lower polymorphism of tetranucleotides and the higher rate of non-specific stuttering (and errors in allele size estimation) in dinucleotide PCR amplifications.

GIS sequenced randomly selected clones using standard DYEnamic™ ET Terminator cycle sequencing reagents on an ABI model 377 DNA sequencer. DesignerPCR 1.03 (Research Genetics Inc.) was used to select PCR primer sequences of similar length and melting temperature for each candidate clone. Primers were then tested under standard PCR conditions. PCR cocktails

contained 6.15 µl water, 1.0 µl of 10X enzyme buffer, 0.4 µl of 50 mM $MgCl_2$, 0.8 µl of 2.5 mM dNTP mix, 0.3 µl of 20 µM forward and reverse primers, 0.05 µl of 5 units $\mu l^{-1}$ BioTaq (Bioline USA Inc.), and 1 µl of 2 ng $\mu l^{-1}$ template DNA. PCR reactions were denatured at 94°C (180 s), followed by 35 cycles of 94°C (40 s), 55°-57°C (40 s), 72°C (30 s), and a final extension step of 72° C (240 s). Annealing temperature varied slightly among microsatellites depending on predicted primer melting temperatures. PCR products were visualized on 1% agarose gels and scored for qualitative presence or absence of a product similar to the expected size (based on the cloned fragment).

**Sequence Analysis of Clones**

Primer pairs designed from 45 clone sequences (GenBank accessions EF106738-EF106783) produced consistent amplification results under the standard conditions above. I searched these clones against other echinoderm sequences (txid: 7586) in the non-redundant nucleotide and protein databases using BLAST 2.2.17 (www.ncbi.nlm.nih.gov/BLAST). I used these results to identify microsatellite clones that were similar to other nucleotide sequences (BLASTn, optimized for somewhat similar sequences) or to gene predictions or identified protein-coding genes (BLASTx). All sets of significant BLASTx matches included an open reading frame from the complete *Strongylocentrotus purpuratus* sea urchin genome. I further characterized these latter similarities by BLASTing individual sequences against the sea urchin database at the Human Genome Sequencing Centre at Baylor College of Medicine

(www.hgsc.bcm.tmc.edu/projects/seaurchin/). I then searched the partially annotated sea urchin genome (annotation.hgsc.bcm.tmc.edu/Urchin/cgi-bin/pubLogin.cgi) for these GLEAN3 genes.

**Species assignment and cross-amplification of microsatellites**

For each microsatellite that successfully cross-amplified in two or more species in preliminary testing, I assigned the cloned sequence to a single species by genotyping each of the individual sea stars in those species for which genomic DNA was used in library construction. I used the GIS standard PCR conditions (above) with the following modifications: I used Tsg DNA polymerase (BioBasic) in place of BioTaq; I added 0.8 µl of 25 mM $MgCl_2$ stock to the PCR cocktail (and adjusted the $dH_20$ amount accordingly); I labelled the 5' end of one forward primer with LI-COR IRDye700 or IRDye800 infrared dyes; I diluted the 100 µM labelled primer stock 1:99 in 10µM unlabeled primer (to reduce background signal); for one trinucleotide (B105) I slightly increased the annealing temperature (58°C) and reduced the $MgCl_2$ concentration (0.5 µl) to reduce shadow banding. These PCR products were resolved in 25 mm 6% acrylamide gels on a LI-COR 4300 genetic analyzer with IRDye-labeled size standards. Fragment sizes were scored from gel images analyzed in Gene ImagIR (LI-COR).

I further explored the cross-amplification of these microsatellites in the Atlantic genus (*Asterina*) that is the sister group to the major Indo-Pacific asterinid clade (Hart *et al.,* 1997; O'Loughlin and Waters, 2004; Waters *et al.,* 2004b; Keever and Hart, 2008). I tested 11 loci that I found to broadly cross-

amplify among Indo-Pacific species. I genotyped a sample of 10 individual *A. gibbosa* for these 11 loci, using genomic DNA extractions from a single population from Wales that had been included in a recent AFLP study (Baus *et al.,* 2005). I used the modified PCR conditions noted above but with a lower annealing temperature (50° C) and fewer amplification cycles (30).

**Population Polymorphism**

I genotyped 15 to 50 individuals of *Patiria pectinifera, Meridiastra calcar, Meridiastra mortenseni, Cryptasterina hystera, Cryptasterina pentagona, Parvulastra parvivipara, Parvulastara vivipara, and Parvulastra exigua* from 3-14 populations from the east and west Pacific oceans (Appendix 1). For all *M. calcar and P. exigua* samples, extractions were conducted using individual tube feet and a simple proteinase K extraction (10 ul PCR-grade $H_2O$ and 2 ul 20mg/ml proteinase K incubated for 1 hour at 85° C) for all populations except for those from Allison Beach (*M. calcar*), Primrose Sands, and Eaglehawk Neck (both *M. calcar and P. exigua*). Samples from these populations, as well as all *P. parvivipara* and *P. miniata* populations, were extracted using a standard CTAB protocol (Addison and Hart, 2004). All other species and populations, including some fill-in samples from *M. calcar,* were extracted using a Quaigen puregene DNA extraction kit. I used the PCR conditions used for species assignment and cross amplification and methods noted above. Loci were chosen on the basis of preliminary surveys of polymorphism, cross amplification, and reliability of PCR

amplification. Samples were genotyped on LICOR and genotypes were scored using the software package Gene ImagIR (LICOR).

I examine microsatellite polymorphism by estimating allele frequencies, mean allele size for each locus, allelic richness, heterozygosity and inbreeding coefficients for each species using Fstat (Goudet 1995), Genepop (Raymond and Rousset 1995), Genodive (Meirmans 2005; Weir and Cockerham 1984), and Arlequin (Excoffier et al. 2005) (see appendices 1-9). Inbreeding coefficients were calculated using the method of Weir and Cockerham (1984), and observed heterozygosity was calculated using the method of Nei et al. (1987). For these estimates I included microsatellite variation for two *Patiria miniata* population samples genotyped by J. Sunday (Keever et al. 2009).

I examined the relationship between amplification patterns and ascertainment bias graphically using the programming language and statistics packages in R (R Development Core Team 2005, www.R-project.org). In these analyses, I used the genetic distance between the focal and non-focal species to identify changes in patterns of microsatellite variation with distance from focal species, which can be used as a proxy for the expected magnitude of ascertainment bias. I used the mitochondrial DNA alignment for all species in the family Asterinidae (O'Loughlin and Waters 2004, Keever and Hart 2008) to calculate genetic distance among all asterinid species pairs using Kimura 2 parameter distance measurement. This method calculates the percentage of nucleotide difference between two haplotypes, taking into account differences in transition and transversion substitution rates (calculated from the data) and

allowing for multiple substitutions per nucleotide site (Kimura 1980). I plotted maximum and mean allele size for each locus against genetic distance from the focal species as a measure of the potential effect of ascertainment bias on variation in allele size between species. Similarly, I plotted genetic distance against both standardized allelic richness and $F_{IS}$ to determine if the ascertainment bias for clone length also affected the underlying levels of within-population allelic variation. I examined mean allelic richness and mean allele size for loci amplified from focal species and from non-focal species, and used Mann-Whitney U tests to determine significance of differences between means. I also examined the association between allele size and genetic diversity by plotting mean allele size against allelic richness.

Finally, I examined breadth of cross-amplification allelic diversity or allele size for a particular locus by plotting breadth of cross amplification (e.g. in 1 2 3 … species) against standardized allelic richness and average allele size for each locus in each species. I also asked whether differences in life history among species affected the relationship between allele size and allelic richness qualitatively by colour coding each data point by its corresponding life history mode.

## .Results

### Microsatellite Characteristics

I obtained positive test results for 45 primer pairs that included five CA repeats (labelled A in Appendix 10), 19 ATG (B), 18 CAG (C), and three TAGA

(D). All CA and TAGA repeats were simple and uninterrupted (with the minor exception of a probable A→C transversion in clone A4). In contrast, seven of 19 clones containing ATG repeats and 12 of 18 CAG repeats included non-repetitive interruptions caused by deletions or substitutions, or consisted of two or three similar repeated motifs, or included a compound repeat consisting of two similar trinucleotides.

BLASTn comparisons of cloned sea star sequences produced four significant matches to other echinoderm nucleotide sequences. One ATG trinucleotide clone (B101) was strongly similar (expectation value $E = 5 \times 10^{-24}$) to the 3' UTR in the genomic DNA sequence for the DNA binding protein *Ap-Zic* from another asterinid, *Patiria pectinifera* (AB231872; Aruga *et al.,* 2006). Two other clones (B202, B236) resembled sea urchin protein coding genes: B202 included an open reading frame strongly similar to the Nk-class homeodomain protein *Sp-Nk7* ($E = 8 \times 10^{-28}$); B236 was similar to the cell surface protein *SRCR* (scavenger receptor cysteine-rich, $E = 5 \times 10^{-5}$). The 3' flanking region of one CA dinucleotide clone (A4) was similar ($E = 2 \times 10^{-9}$) to the flanking sequence of a CA dinucleotide from *Acanthaster planci* (AB220018). Two clones (C114, C227) showed highly significant ($E = 10^{-129}$) nucleotide similarity to each other. These sequences might represent two alleles at a single locus, but the clones showed considerable sequence divergence (48 nucleotide substitutions, 11.8%).

BLASTx comparisons to coding sequences produced three highly significant matches to sea urchin genes. All matches involved ATG or CAG repeats. Two of these strong similarities were to the predicted protein sequences

from some sea urchin open reading frames to which I also found significant nucleotide sequence matches (B202, *Sp-Nk7*; E = 7 x $10^{-17}$) (B236, *SRCR*; E = 1 x $10^{-12}$). A third clone (B114) included an open reading frame similar (E = 1 x $10^{-5}$) to a sea urchin adhesion protein in the extracellular matrix (*anosim-1* or *KAL-1*, defective in human Kallmann syndrome). This locus was also a highly similar match (E = 1.3 x $10^{-12}$) to a recent unannotated EST (DB439856) cloned from 45-hour-old embryos of *Patiria pectinifera* (K. Tachibana, Y. Suzuki, T. Shin-i, Y. Kohara, M. Sugano, T. Kishimoto; unpublished data).

**Species Assignment of Loci**

Thirty-nine of 45 clones (0.87) could be unambiguously assigned to one of eight species (and in some cases to individual sea stars in the genomic DNA pool). Number of clones assigned to each species varied from two to ten (Appendix 11). A majority of clones (25) were isolated from one clade consisting of the sister genera *Meridiastra* + *Patiria* (Appendix 11). Six other microsatellites could not be reliably assigned to individual species either because the cloned allele size was found in individuals from two or more species (B106, B202, B234, B236) or because the cloned allele size was not found among any individuals that were genotyped (B222, C112).

**Cross-Amplification in *Asterina***

Five of 11 microsatellites that were broadly cross-amplified among Indo-Pacific species could also be amplified in the outgroup *Asterina gibbosa*. The

success or failure of these cross-amplifications was not obviously associated with repeat motif or interruptions, species to which the clone was assigned, or the number of Indo-Pacific species in which the microsatellite could be amplified. All five of the successfully cross-amplified markers were fixed for a single allele size in our small sample of 10 *A. gibbosa* individuals. This lack of variation is surprising: three of these microsatellites (B202, C8, C204a) are known to be polymorphic in Indo-Pacific species (Appendix 2, see below), and previous studies show within-population variation in dominant AFLP fingerprints of *A. gibbosa* (Baus *et al.,* 2005).

**Population Polymorphism**

Within each population sample I found 1-26 alleles per microsatellite (Appendix 2) and a broad range of allelic richness values (1-11) (Appendix 4) and observed (0-0.82) (Appendix 5) and expected (0-0.88) heterozygosity (Appendix 5). Among 9 species and 113 pairs of loci I found 3 pairs of microsatellite loci in linkage disequilibrium (Appendix 7). Interestingly, of these 3 pairs of loci, none were found to be in linkage disequilibrium in more than one species. In each of 9 species 1-7 loci showed high inbreeding coefficients and significant heterozygote deficits relative to Hardy-Weinberg equilibrium (Appendix 6). I found the greatest number of alleles at locus C112 in *Meridiastra calcar* and the highest inbreeding coefficients at A110 in *Parvulastra parvivipara*. Both of these loci cross-amplify in more than one species studied. The focal species for A110 is indeed *Parvulastra parvivipara*, while the focal species for C112 is ambiguous.

**Polymorphism and Cross-Amplification Patterns among Indo-Pacific Species**

I selected the largest and smallest populations for each species to test cross amplification patterns and the potential effect of ascertainment bias on polymorphism.

When mean allele size and maximum allele size were plotted against genetic distance from the focal species, only one locus (C219) was found to have significantly decreasing allele size with increasing genetic distance. ($R^2$ = 0.98, P =0.0043) (Figure 3.1ab). Similarly when I plotted genetic distance against standardized allelic richness and $F_{IS,}$ I found no significant association between either of these measures and genetic distance from focal species for any locus. Plots of allelic richness are shown (Figure 3.1de).

I found significantly higher allelic richness in loci amplified in focal species compared to cross-amplified loci in non-focal species using a Mann-Whitney U test (U=248, P =0.02). When I conducted the same test using mean allele size in focal vs. non-focal species there was no significant difference in mean size between loci amplified in focal species or those amplified in non-focal species (w=114, P =0.5) (Figure 3.1cf). The difference between those two results suggests that the lower allelic diversity within cross-amplified microsatellite samples is probably not caused by ascertainment bias associated with the isolation and cloning of longer genomic DNA fragments in the focal species.

I also plotted the cross amplification breadth of each locus against allelic richness and average allele size. There was no significant relationship between

the cross-amplification breadth and either allelic richness or allele size (Figure 3.2) but there was a clear tendency toward higher allelic richness in species with planktonic larval development for both broadly-crossamplifiable loci and loci that were amplifiable in only one or two species.

## .Discussion

The among-species and among-clade differences in the number of cloned microsatellites highlight one of the potential pitfalls of our microsatellite isolation approach using pooled genomic DNA. The pooling approach helps to ensure that all microsatellites from different species are isolated simultaneously under identical enrichment conditions (and at 1/N the cost for libraries developed for each of N species). However, libraries enriched for microsatellites from single species (at higher cost) could have allowed me to find many more markers from *Cryptasterina* and *Parvulastra* species and avoid the potential effects of using mostly cross-amplified markers in population analyses of those species (see below).

Many published microsatellite descriptions (primer notes) do not specifically include BLAST sequence comparisons to known genes, but I found some broadly cross-amplifiable microsatellites that I could confidently identify with coding sequences. Several empirical studies and reviews have noted broader cross-amplification among microsatellites in genes identified from ESTs than among microsatellites isolated from genomic DNA (see Bouck and Vision, 2007). Only two loci in our study are associated with coding sequences (B202

and B236). One locus (B202) was amplified in all 9 species and this locus showed no qualitative difference in measures of allelic richness or Fis values compared to other loci in the data set. The other locus (B236) was only amplified in one species. For B236 and B202 I was unable to identify the clone source because several species shared the cloned allele size. This ambiguity could be the result of size constraints on these loci, or selection for the cloned fragment size caused by functional constraints on the gene product (e.g., Li *et al.,* 2002, 2004).

Microsatellites often show high inbreeding coefficients that may be caused by segregation of null alleles. This can be due to evolutionary divergence of flanking sequences that include the PCR primer sites (Selkoe and Toonen, 2006). I found many examples of high inbreeding coefficients associated with significant heterozygote deficits, which could be caused by the evolution of null alleles (Appendices 5 and 6). High rates of flanking sequence evolution and high frequencies of microsatellite and allozyme null alleles may have interesting correlations with biological and demographic processes (Zouros and Foltz, 1984; Addison and Hart, 2005).

One goal of our microsatellite development was the discovery of broadly cross-amplifiable markers. Cross-amplification varied considerably among loci and I found little evidence that either genetic distance from the focal species or breadth of cross amplification was associated with allele size. I found some evidence that allelic richness was associated with amplification in focal vs. non-focal species. However, I found no association between breadth of cross-

amplification or genetic distance from focal species for either $F_{is}$ values or allelic richness. Compared to the results of ascertainment bias frequently observed in microsatellite cross-amplification studies, I find a significant difference in allelic richness but not in average allele size in focal versus non-focal species.

Previous studies postulated that these two measures were causally linked (i.e., greater allelic richness due to higher rates of mutation in species with longer allele sizes), but our study finds that both measures do not vary equally with amplification patterns (Hutter et al. 1998). Furthermore, I find no associations between average allele size and allelic richness in our dataset indicating that the two are not tightly coupled. In general, these results suggest that microsatellite variation within asterinid population samples is not strongly affected by artefacts associated with ascertainment bias and cross-amplification, and that life history differences among species (and the associated differences in the magnitude of inbreeding and genetic drift) are more important determinants of allele size variation (e.g., Fig. 3.2B).

One possibility for this phenomenon is that an intrinsic property such as life history is driving the patterns of allelic richness. The data shows that the results are driven by a combination of factors. In some cases such as for loci C113 and C219, differences in life history between planktotrophic focal species and related livebearers result in a drastic reduction of allelic richness. In other cases (B227) a reduction in allelic richness occurred in the related species. There are other cases such as microsatellite A110 where there is no drastic change in allelic richness with life history or distance from the focal species. In

agreement with life history driving the patterns of allelic richness but not average allele size, are the plots of breadth of cross-amplification (Figure 3.2). In these data, both measures of allele size and allelic richness show no clear pattern of association with breadth of cross-amplification, but when the data points are colour coded by life history there is an obvious visual association between allelic richness and life history and no association for allele size and life history. The association of allelic richness with life history and not breadth of cross amplification further suggests that another factor, possibly life history, is driving patterns of allelic richness.

Species differences in microsatellite mutation rate may also contribute to these allelic diversity differences. Studies of sequence evolution and microsatellite cross-amplification in humans and chimpanzees have shown that directional differences in allele size and allelic richness are driven by both ascertainment bias and differences in mutation rates between species in the microsatellite flanking sequence (Vowles and Amos 2005; Ellegren 2000). The majority of our loci were cloned from *Meridiastra calcar* and *Patiria miniata*. These large-bodied, gonochoric species have high male and female gamete production compared to small-bodied self-fertilizing hermaphrodites in other genera (*Parvulastra, Asterina, Cryptasterina*), and may have higher mutation rates as well. If these species have intrinsically higher mutation rates than other asterinid species then global measures of polymorphism such as that in Figure 3.1 may be biased by the underlying mutational variation. The potential for these processes to be operating also argues for the development and use of improved

standardization methods in estimates of population differentiation (Hedrick, 2005; Meirmans, 2006) in population analyses using these markers.

## General Conclusions

The data and analyses from this chapter thoroughly examine the amplification patterns of the asterinid microsatellite markers. I find that ascertainment bias does not appear to pose a substantial problem for analysis of population genetic patterns using cross-amplification of microsatellites across species. However, I do find that levels of genetic diversity (measured as allelic richness) differ significantly among loci in a pattern not associated with focal species ascertainment bias. This result suggests that the markers can be used for among-species comparisons of population genetic structure that will mainly reflect the population biology of the organisms and not the process for developing the markers.

**Figure 3.1 Relationship between allele size (a,b,c), allelic richness (d,e,f) and cross amplification patters. Colour represents repeat type CAG = blue, CA = green, ATG = red, CAA = yellow. Parts a and c represent mean allelic richness and allele size plotted against genetic distance and parts b and e represent maximum allele size and allelic richness plotted against genetic distance. Parts c and f represent the difference between allele size and allelic richness in loci amplified in the species in which they were cloned in (focal) or in species for which they cross-amplify (non-focal), unknown represents loci for which the cloned focal species was unknown.**

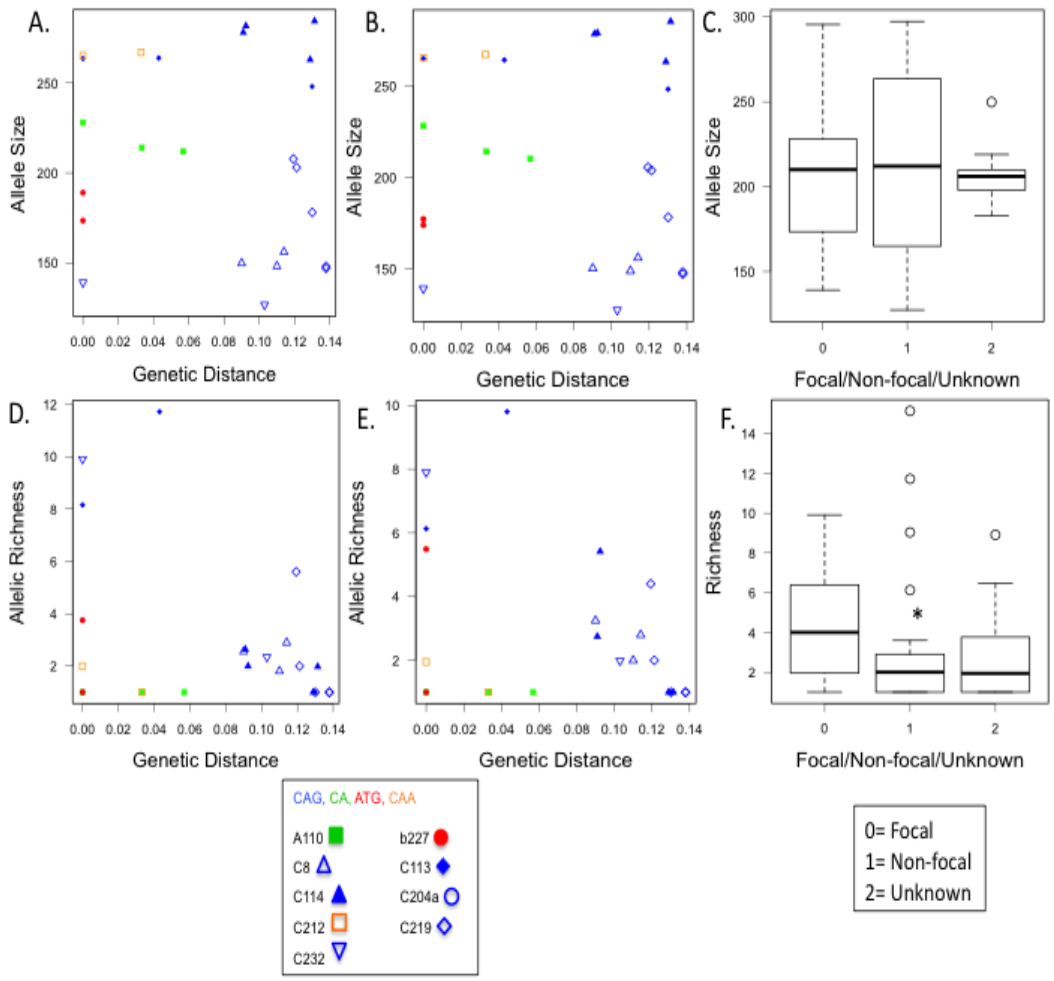
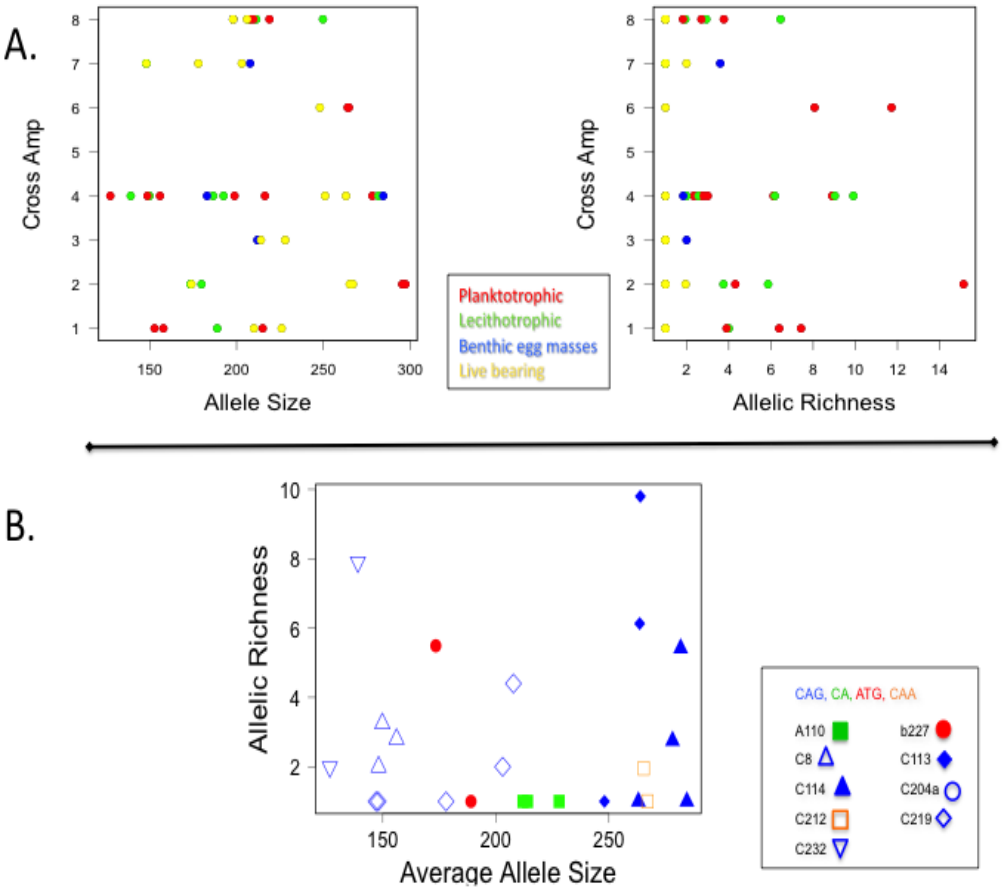


**Figure 3.2 The relationship between breadth of cross amplification (number of species that a marker amplifies in) and both mean allelic richness and mean allele size for that marker (A). In part A colours represent life history modes red = planktotrophic, green = lecithotrophic, blue = benthic egg masses and yellow = live bearing. Part B shows the relationship between allelic richness of microsatellite loci and allele size. Each point represents a species measure for a particular locus. In this section (B) colour represents repeat type CAG = blue, CA = green, ATG = red, CAA = yellow.**



# Chapter 4 **ANALYSIS OF POPULATION GENETIC STRUCURE IN ASTERIND SEA STARS WITH MULTIPLE MODES OF DEVELOPMENT**

## .General Introduction

In this chapter I examine patterns of population genetic differentiation in 8 species of asterinid sea star including *Patiria pectinifera, Meridiastra mortenseni, Meridiastra calcar, Cryptasterina pentagona, Parvulastra exigua, Parvulastra parvivipara, Parvulastra vivipara,* and *Cryptasterina hystera.* I examine these patterns first in the context of predictions based on life history differences among species and secondly in the context of previously discovered extrinsic factors that may interact with life history to generate population genetic structure. I then use the results from this chapter to conduct a comparative analysis of the correlation between life history mode and population genetic variation (Ch. 7).

## .Introduction

Population genetic variation can be generated by a species' intrinsic life history characteristics, or extrinsic factors such as contemporary or historical features of a species ecological or oceanographic environment. Understanding the relative contribution of these forces is a fundamental question in molecular ecology (Grosberg and Cunningham 2001). In the absence of any strong historical or contemporary barrier to gene flow, intrinsic factors such as a species'

dispersal ability or mating system may drive patterns of population genetic variation. However, extrinsic barriers to dispersal and temporal changes in their effectiveness can weaken the expected correlation between life history variation and patterns of population genetic differentiation (Jablonski 1986, Jablonski and Raup 1995, Wares and Cunningham 2001).

Comparative studies of closely related marine species have found that life history traits vary in their influence on population genetic structure. Population genetic research suggests that in many cases larval dispersal mode (Duffy 1993, Hunt 1993, Hoskin 1997, Lee and Boulding 2009), or mating system (Edmands and Potts 1997) predicts levels of within and between population genetic variation. Often life history influences the way that a population responds to extrinsic forces such as historical vicariance (Arndt and Smith 1998). Numerous counter examples have shown that historical vicariance, oceanic current patterns and differences in other aspects of life history or ecological niche can override the influences of larval form and mating system in predicting similarities or differences in population genetic variation among closely related species (McMillan et al. 1992, Kyle and Boulding 2000, Hickerson and Cunningham 2003, Marko 2004, Ayre 2009).

The distribution of genetic variation among individuals, populations and regions compared among closely related species that differ in life history strategy can shed light on the contribution of intrinsic factors to shaping genetic differentiation. If species with similar life histories, living in different environments and ecological niches, have similar levels of within- or among-population genetic

variation then the species' mating systems or larval dispersal abilities may be more important than extrinsic factors in structuring populations. Alternatively, similar levels of within- or among-population genetic variation for sympatric species with different life histories would argue against the primary importance of those life history differences (and may argue in favour of extrinsic factors) in predicting patterns of differentiation. Moreover, comparing these patterns of differentiation among closely related species can account for similarities or differences in population genetic structure that arise due to shared ancestry rather than differences in intrinsic dispersal ability or extrinsic factors.

In this chapter I examine patterns of population genetic differentiation among species of asterinid sea stars, which have evolved similar modes of development (larval dispersal and mating systems) in parallel. If life history is primarily driving patterns of population genetic variation then species with planktonic larvae (feeding or non-feeding) should have higher within- and lower among-population genetic variation relative to species with benthic development (either in egg masses or inside the parent). Similarly, gonochoric asterinid species should have higher within- and lower among-population genetic variation relative to hermaphroditic species.

I compare both sympatric species pairs with different life histories (*M. calcar* and *P. exigua*) and allopatric pairs with similar and different life histories (*P. parvivipara* and *P. vivipara*) (see chapter 1), and use these comparisons to test predictions about the role of intrinsic dispersal ability as well as extrinsic properties of a particular species' environment in producing population genetic

structure (Ch 1). I will first investigate the role of larval form and mating system in generating within-population genetic variation and patterns of differentiation among populations among asterinid species. Secondly, I will consider information on extrinsic factors that may influence population genetic structure either independently of or in addition to life history variation.

**Previous Findings and the Role of Extrinsic Factors**

Historical and contemporary extrinsic characteristics within each species' range can interact with life history to generate patterns of within and between population genetic variation that are unique to a particular region (egg. Marko et al. 2010). I examine patterns of population genetic differentiation, keeping in mind predictions generated from previous findings on population genetic and phylogeographic structure that implicate extrinsic factors in shaping the genetic signature (Table 4.2). Specifically, I examine phylogeographic patterns in Australia and New Zealand in regions of overlap with my study species and I use this information to examine specific predictions about how life history might interact with both historical and contemporary extrinsic factors (Figure 4.1).

High variation in seasonal sea surface temperatures, strong eddies, and upwelling at 32-33° south latitude of the East Australia Current (EAC) along the coast of New South Wales (NSW) have been implicated in the isolation of populations despite the presence of long distance dispersal in the sea urchin *Centrostephanus rodgersii* (Banks et al. 2007*)*. This species has a feeding planktonic larva that can exist in the plankton for up to 4 months before settling.

High levels of gene flow are found between New South Wales and New Zealand, and at the same time high levels of heterogeneity are found among populations along the coast of NSW. Spatial oceanographic models of genetic differentiation in this species along the coast of NSW, suggest that genetic structuring is greater in areas of greatest variability in sea surface temperature.

There are three common phylogeographic breakpoints span four Australian States, including New South Wales, Tasmania, Victoria and South Australia (SA). The regions separated by these points correspond to the three main Australian biogeographic provinces in southern and eastern Australia (Flindersian, Maugea and Peronia) (Bennett and Pope 1953). I expect that patterns of differentiation that I see may correspond to these regions as they have in many other studies (Figure 4.4) (egg. Waters 2008). First, species that range throughout NSW and South Australia and whose ranges often encompass Tasmania, show strong phylogeographic structure between populations east and west of Bass Straight, a region exposed during low sea level stands in the Pleistocene (Waters 2003, Dawson 2005, Waters et al. 2006, York et al. 2008). The second, less significant break is among populations on Tasmania vs. mainland Australia. Weakening of the EAC during the Pleistocene has been implicated in this disjunction (Fraser et al. 2009). Lastly, a recent community study in eastern Australia by Ayre et al. (2009) showed that species with planktonic larvae were more differentiated on either side of a contemporary barrier at Ninety Mile Beach then were species with non-planktonic larvae

(including *P. exigua*) and both habitat and life history were suggested to be involved with different responses to ecological conditions.

Species found on the south coast of Australia near the Great Australian Bight are subject to highly seasonal current patterns, and in some regions isolation due to eddies formed by the Leeuwin and Flinders Currents (Middleton and Bye 2007). Two studies of population genetic structure in this region suggest that eddies from the Leeuwin current cause populations in the northeast region of the Great Australian Bight to be genetically isolated. Populations of bottle nosed dolphins and King George Whiting showed strong recruitment patterns to populations within Streaky Bay South Australia (Fowler et al. 2000, Bilgman et al. 2007). Although, the effects of oceanography probably are important in these systems, behavioural factors such as mate choice, feeding and philopatry are likely also important factors shaping population structure.

Species found north of 23° south latitude on the East Coast of Australia in Queensland are subject to two important biogeographic boundaries caused by convergence of currents. The first of these boundaries is formed by the convergence of the South Pacific anticyclone gyre (Hiri) and the Southern Equatorial Current along the north east Coast of Australia, at about 15° south latitude (Figure 4.4). This convergence zone acts as a phylogeographic break in caridean shrimp species (Haig et al. 2010). The second zone of convergence happens near 23° south latitude where cold water from the east mixes with the warm equatorial waters of the northern EAC (Burrage et al. 1996). This boundary is also the southern extent of the Great Barrier Reef, which

corresponds to the range limit of another asterinid sea star species in this study, *Cryptasterina hystera*.

A large proportion of research on Great Barrier Reef (GBR) taxa has been in fish species that can potentially disperse as adults. One particular study of a fish species in the family Pomacentridae that lacks a planktonic phase shows high levels of genetic differentiation among populations in different regions of the GBR (Doherty et al. 1994). Another study on allozyme variation in seven fish species on the GBR found high levels of heterogeneity (including fixed allelic differences within species) among reefs (Doherty et al. 1995). This research suggests that species that are benthic at some point in their life cycle or that lack a planktonic larval phase but that range between island and mainland populations may exhibit high levels of population genetic differentiation.

For coral species living on One Tree Reef at the southern extent of the Great Barrier Reef, life history doesn't always reliably predict levels of population genetic differentiation (Miller and Ayre 2008). *Goniastrea favulus* is a hermaphroditic coral species with a benthic larval form and *Platygyra daedalea* is a gonochoric coral species with planktonic larvae. These two species exhibit similar levels of pairwise population genetic differentiation and fixation indices, but differences in levels of allelic diversity and heterozygosity within populations. In this case *P. daelalea* shows evidence of non-sexual reproduction that could be responsible for this seemingly anomalous result. However, similarities in recruitment patterns, or environmental heterogeneity between the two species or

selection on microsatellite markers used in this analysis could also be driving patterns of differentiation (Ayre and Miller 2004)

On the North Island of New Zealand phylogeographic studies have shown genetic differentiation between North vs. South and East vs. West Coast populations.. In a study of the brittlestar *Amphipholis squamata* population structure was found mainly between the North and South Islands of New Zealand, however the only population sampled from the south end of he North Island grouped with populations on the South Island rather than grouping with the remainder of populations sampled on the North Island (Spooner and Roy 2002). In another study Waters and Roy (2004) found that there was a significant amount of genetic structure among populations of an asterinid sea star, *Patiriella regularis,* on the east and west sides of the North Island. They suggested that unique currents such as the East Auckland and West Auckland, working in combination with the D'Urville and East Cape currents caused differentiation between east and west.

My research examines the population genetic structure of sea stars of the family Asterinidae. I use patterns identified in previous species to develop predictions for geographical structuring of population genetic variation (see Table 4.2) in the asterinid species, and tests these patterns specifically in AMOVA analyses. Although I am ultimately interested in the relationship between life history and population genetic structure, the predictions and analyses related to the influence of extrinsic factors on the population genetic structure of asterinids

provide likely alternatives, when life history alone cannot explain all of the variation.

I conduct a series of comparisons to unpack the population genetic results in asterinid sea stars by selecting specific comparisons among species that explore the relative influence of evolutionary constraints, life histories and extrinsic influences on population genetic variation. First I compare species with similar life histories living in different geographic regions. If these species have similar levels of within- and among-population genetic variation then life history may be a stronger predictor of patterns of population genetic variation. Alternatively, if these species have different patterns of population genetic variation then the specific characteristics of each species' geographic region may be driving patterns of genetic variation. Secondly, I compare species with different life history strategies living sympatrically. If these species show similar patterns of within- and among-population genetic variation then characteristics of their shared geography could be driving patterns of population genetic structure. However, if patterns of population genetic variation differ among these species, in the direction predicted by their life history differences, then intrinsic properties could be more important in predicting patterns of population genetic variation. Finally, I investigate individual sister species pairs that share a similar life history strategy or where sister species differ in their life history strategies. If patterns of population genetic variation are similar among sister species with different life history strategies then phylogenetic signal (or factors related to phylogenetic signal including geographic range) among these species may be driving patterns

of population genetic variation. However, if sister species that differ in life histories show different levels of population genetic variation in the direction predicted by life history then phylogenetic signal may be less important in driving patterns of population genetic variation among these species.

## .Methods

DNA extraction protocol and PCR conditions were the same as those described in Chapter 3. Samples were run on a LICOR model 4300 Genetic Analyzer. Allele sizes were estimated using Gene ImagIR (LICOR). Samples for which results were ambiguous or equivocal were rerun under the same conditions to assure consistency.

The majority of tube foot samples for *M. calcar, P. exigua, P. parvivipara, C. hystera* and *C. pentagona* were collected in Australia in Fall 2005. During this trip I recorded the names, locations, and dates of sampling (Appendix 1). Tube foot samples for *M. mortenseni* and *P. pectinifera* were collected by collaborators between January 2006 and October 2007. Samples of *P. vivipara,* which is federally listed as a vulnerable species were collected by Maria Byrne in 1991 and stored at -80°c. All tube feet were preserved in 95% ethanol in the field and samples were transported to the lab for analysis.

DNA extraction protocol and PCR conditions were the same as those described in Chapter 3. Samples were run on a LICOR model 4300 Genetic Analyzer. Allele sizes were estimated using Gene ImagIR (LICOR). Samples for which results were ambiguous or equivocal were rerun under the same conditions to assure consistency.

## Genetic Variation Within Populations

I calculated allele frequencies and allele counts in Genodive (Meirmans 2004). I plotted allele size frequency distributions for each species, population, and locus in the statistics package R (Development Core Team 2005, www.R-project.org) to determine whether the loci in this study have evolved according to a stepwise model of microsatellite mutation or whether these loci appear to have mutational patterns that fit another model of microsatellite substitution. Tests for normality were conduced using Shapiro-Wilk normality tests and plotting the data on a QQline plot to test for linearity (Shapiro and Wilk 1965).

For each population sample, I measured allelic richness at each locus using the program Fstat, which standardizes richness to the smallest population sample size (Goudet 1995). I calculated inbreeding coefficients and carried out tests for Hardy-Weinberg equilibrium within populations using the Wier and Cockerham method (1984) in Genodive (Meirmans 2004). Observed heterozygosity was measured using the program Genepop (Raymond and Roussett 1995). I estimated $\Theta_H$, the effective number of homozygotes (an index of effective population size), using the program Arlequin (Excoffier et al. 2005).

I measured linkage disequilibrium among loci for each species and population using the program Genepop (Raymond and Roussett 1995). Linkage among loci within populations as well as a global test of linkage was tested using the G-test likelihood ratio statistic with 100 batches and 1000 iterations per batch.

**Genetic Differentiation Among Populations: Bayesian Clustering**

I used a Bayesian clustering model that assigns individuals to population clusters based on genotype associations in STRUCTURE (Pritchard et al. 2000). This program calculates the likelihood of observing K clusters of individuals in a data set based on linkage disequilibrium between genotypes at pairs of loci and deficits of heterozygote genotypes for single loci in a sample. STRUCTURE uses a Markov chain Monte Carlo (MCMC) search of the parameter space to derive a posterior distribution of genotype/population combinations.

I conducted two runs for each species. The first was a trial of 1,000,000 iterations, and I carried out 1 independent run for each of k-1 populations (where K is equal to the number of sites sampled for each species). This model was run with admixture and uniform priors. For each species I conducted two more independent runs for each of 1-k (k = 5 *M. calcar, P. exigua, C. pentagona,* and *C. hystera* and k = 3 for *M. mortenseni, P. pectinifera, P, parvivipara,* and *P. vivipara*) with 5,000,000 iterations of the data and a burnin of 500,000. Convergence on the set of population clusters with the highest probability was assed using the $\Delta$K test of Evano et al. (2005)

**Genetic Differentiation Among Populations: Hypothesis Testing**

I estimated differentiation between pairs of populations using the $\Phi_{ST}$ measurement of Weir and Cockerham (1984) in Genodive (Meirmans 2004). A separate pairwise $\Phi_{ST}$ analysis was run for each locus for each species independently. Loci were combined in each species to calculate an overall

multilocus measure of pairwise population genetic differentiation. Genetic differentiation between populations was standardized to one (Meirmans 2006, Hedrick 2005). Different allelic richness and genotype frequencies within populations can affect the maximum observable level of genetic differentiation between populations and render levels of differentiation less easily comparable across loci and species. The standardization method allows for the comparison of measures of population genetic differentiation among loci and species that have very different levels of within-population genetic diversity. Significance of population genetic differentiation was tested using the G-test statistic in Genodive and alpha levels were adjusted using a Bonferroni correction.

I used analysis of molecular variance (AMOVA) (Excoffier et al. 2005) to characterize overall differentiation among populations within each species. This method investigates how genetic variance is partitioned into differences within individuals (i.e., heterozygosity), among individuals within populations, among populations within phylogeographic groups, and between two or more of these population groups. Significance tests used 10,000 permutations of the data to determine measures of differentiation significantly different from zero.

I examined predictions about plausible phylogeographic structure by grouping populations within each species in accordance with previous phylogeographic findings from the region that each species inhabits (see short review in introduction above) (Table 4.2). If I find concordance with asterinid population genetic differentiation and any of the patterns reviewed previously (see introduction to Chapter 4), then it is possible that the extrinsic factors

implicated in these studies could also be working in the Asterinidae. Population abbreviations in this section can be found in Figure 1.2 and Appendix 1.

For *M. calcar* and *P. exigua* I tested three AMOVA models: a two-group model that compared Tasmania (TAS) populations to all other New South Wales (NSW) and South Australia (SA) populations (Fraser et al 2009); second, an east-west split in which New South Wales and Tasmania populations were separated from South Australia populations (Waters et al. 2003, Dawson 2005, Waters et al.2006 and York et al. 2008 ); and lastly, a three-group model, where New South Wales, Tasmania, and South Australia populations were all in separate groups (Bennett and Pope 1953). For *C. hystera* I tested a two-group model that compared populations on One Tree Island on the Great Barrier Reef to those on the mainland coast of Queensland (Doherty et al 1995). For *C. pentagona* I tested two different two-group models: one that compared two northern samples (TV,BB) to all other populations to the south; and another comparing the northernmost population (BB) to a second group of all populations to the south (including TV). I based these alternative models on previous descriptions of two biogeographic breaks, one at 23° south and another at 15° south (Burrage et al. 1996, Haig et al. 2010). For *M. mortenseni,* I tested two different two-group models: one that compared populations from the north and south regions of the North Island of NZ; and one that compared populations from the east and west coasts of the North Island (Spooner and Roy 2002 and Waters and Roy 2004). For *P. parvivipara,* I tested one two-group model (based on the pairwise population $F_{st}$ patterns) that compared populations from Smooth Pools

to all other populations (Fowler et al. 2000, Bilgman et al. 2007). I did not carry out AMOVA analyses of *P. pectinifera* in Japan or *P. vivipara* because the narrow sampled range prevented any meaningful hypothesis of differentiation. I also carried out an additional AMOVA for each species without phylogeographic groupings in order to estimate global population differentiation relative to variation within individuals and within sampled populations.

Wherever the STRUCTURE results suggested a population grouping that was not also considered in the previously described AMOVA analyses based on phylogeographic predictions, I conducted additional AMOVA analyses that used population groupings based on the heuristic search results from STRUCTURE (Pritchard et al. 2002).

**Analysis of Isolation By Distance (IBD)**

Geographic distance among populations for each species was calculated using the Line function in Google Earth (2010 Google). The distances inferred were found using the shortest possible path from one population to the next. This estimate involved sometimes drawing lines though landmasses, however many species in this study exist along a linear stretch of coastline and it was not necessary to calculate distance through a landmass. Three exceptions included *M. calcar, P. exigua,* and *M. mortenseni* and in these cases linear distance drawn through landmasses, rather than traversing the coastline would be a conservative estimate of geographic distance between populations. The GPS waypoint (when available; see Appendix 1) was entered into Google Earth and the corresponding

population was labelled on the map. When GPS coordinates were not available a search was conducted for the population by name. Linear distances (km) between all possible pairs of populations for each species were recorded in a distance matrix. The matrices of geographic distances were compared to matrices calculated using the genetic differentiation measure of Weir and Cockerham (1984). A Mantel test of the matrix correlation between distance and differentiation was conducted using Genodive (Meirmans 2004).

## .Results

I sampled 13-68 individuals at 5-7 loci, for 3-13 populations for 9 species of asterinid sea stars (Appendix 1). Much of the DNA extraction, and all of the microsatellite genotyping, for *P. miniata* was conducted by Jennifer Sunday. These data are included in much of the comparative analysis and some of the appendices of population structure for completeness and in order to use this species as another data point for the comparative analysis of population structure and life history in chapter 7.

DNA extraction protocol and PCR conditions are described in Chapter 3. Species, populations and individuals sampled can be found in Appendix 1.

### Size Frequency Distribution

Several loci appeared (by eye) to have normally distributed allele size frequency distributions (Appendix 9). A Shapiro-Wilk normality tests found none of the loci to be normally distributed, nor did any of the loci conform to normality

using a QQline plot. About 46% of the distributions appeared (by eye) to be approximately normally distributed (e.g.. C219 *P. exigua,* C113 *P. pectinifera*), while 7% appeared bimodal (B227, *C. hystera*), 17% left skewed (e.g.. C8 *M. calcar* or A110 *P. parvivipara*), and ~30% right skewed (A110 *P. exigua*). Notable patterns include B227 with a strongly bimodal allele size distribution in both *C. hystera* and *C. pentagona*. The loci that do appear approximately normally distributed tend to be those from highly polymorphic species (*M. calcar* C232), or a highly polymorphic locus in a species with few polymorphic loci (*P. exigua* C219).

**Analysis of Linkage**

After Bonferroni correction for multiple tests, only three pairs of loci were found to be in linkage disequilibrium (Appendix 7). Two of the three pairs were found in *M. calcar* (B236 vs. C204a, C114 vs. C204a), while the third was found in *P. pectinifera* (B202 vs. C113) (Appendix 7). These pairs of loci were amplified in other asterinid species and were not found to be in linkage disequilibrium. These results rule out physical linkage as the cause of associations among most loci, and instead suggest that any associations are more likely the result of population genetic structure.

**Genetic Variability Within Populations**

Allelic Richness

Results of allelic richness can be found in (Appendix 4). Allelic Richness ranged fom 1 (from multiple populations and species) to 15.4 (in *M. mortenseni*). Overall allelic richness was higher for species with planktonic development and separate sexes than for species with benthic development, live bearing and hermaphroditism.

*Planktotrophic Species* – In *Meridiastra mortenseni,* allelic richness varied from 1.99 (locus c232 Wangarei New Zealand) to 8.91 (locus c112 Mission Bay New Zealand). In *P. Pectinifera* allelic richness ranged from 1.82 (locus B202 in Futagojima Japan) to 15.14 (locus B201 in Asamusi Bay Japan).

*Lecithotrophic Species* – In *M. calcar* allelic richness ranged from 2.0 (B202 Gordons Bay New South Wales) to 10.19 (C232 Balmaural Beach New South Wales). In *C. pentagona* allelic richness ranged from 1 (locus C219) to 6.72 (locus B231 Arlie Beach Queensland).

*Benthic Egg Mass Laying Species* – In *P. exigua* allelic richness ranged from 1 (all loci) to 5.59 (locus C219 in Balmaural Beach New South Wales)

*Live Bearing Species* – Allelic richness ranged from 1 to 2 (locus C212) in *P. parvivipara.* In *C. hystera* all populations were fixed for a single allele at all loci and thus allelic richness was 1 for all populations and loci. Finally in *P. vivipara* allelic richness ranged from 1 to 2 (locus c219).

Heterozygosity

Overall heterozygosity varied from 0 (for A102, A110, B202, B227, C104, C112, C113, C114, C212, C219, and D8 in multiple species and populations) to

0.79 (C232 for *M. calcar* at Little Bay NSW), and was higher for species with planktonic larval development and separate sexes. Heterozygosities reported by species locus and population can be found in Appendix 5.

*Planktotrophic Species* – Heterozygosity ranged from 0.02 to 0.86 in *P. pectinifera* and the average total heterozygosity for all populations and loci was 0.34. Heterozygosity in *M. mortenseni* ranged from 0.09 to 0.75, with a total heterozygosity of 0.33.

*Lecithotrophic Species* – Heterozygosity ranged from 0.00 to 0.72 in *C. pentagona,* with a population average total heterozygosity of 0.20. In *Meridiastra calcar* heterozygosity ranged from 0.00 to 0.77 and the average total heterozygosity for all populations and loci was 0.42.

*Benthic Egg Mass Laying Species* – Heterozygosity ranged from 0 to 0.61 in *P. exigua,* with a total average heterozygosity for all populations and loci of 0.08.

*Live Bearing Species* – In *C. hystera* all populations had zero heterozygosity because polymorphism was found only between populations. Similarly, heterozygosity was zero in all *P. vivipara* populations: some populations included >1 allele (unlike *C. hystera*), but different alleles always occurred in different individuals (as homozygotes). *Parvulastra parvivipara* showed variable heterozygosity among populations: individuals from the population Smooth Pools in Streak Bay Australia had higher levels of heterozygosity (0.05 vs. 0) relative to other populations in this species that all had heterozygosities of 0.

$\underline{F_{is}}$

Overall, $F_{is}$ (deviation from Hardy Weinberg Equilibrium within populations) ranged from -0.17 in (at B202 for West B in *M. calcar*) to 1 in multiple populations of *P. vivipara and P. parvivipara* for C232 and C219 respectively, and in one population of *P. exigua* (Shell Harbour 2 NSW) at locus A110. Many populations and species had $F_{is}$ values that could not be estimated due to lack of polymorphism for the particular marker at the particular population (e.g.. C112 in *P. exigua,* CL and TP). If one assumes that these markers lack polymorphism due to non-random mating among individuals then $F_{is}$ values are essentially one as well (Appendix 6).

*Planktotrophic species* – All populations of both *M. mortenseni* and *P. pectinifera* had $F_{is}$ significantly greater than zero over all loci ($0.000<P<0.001$).

*Lecithotrophic species* – Significant overall deviations from Hardy Weinberg were found in 11 of 14 M*. calcar* populations and 4 of 7 *C. pentagona* populations ($0.000<P<0.002$).

*Benthic Egg Mass Laying Species* – In *P. exigua*, where $F_{is}$ could be calculated, only 2 out of 11 populations showed an overall deviation from Hardy Weinberg Equilibrium ($F_{is}$) ($0.000<P<0.002$).

*Live Bearing Species* – For all live bearing species that showed within-population polymorphism (where $F_{is}$ could be calculated), populations showed a significant overall deviation from random mating ($0.000<P<0.002$).

Effective Population Size Theta (H)

Indices of effective population size, measured as theta (H) (the effective number of homozygotes relative to heterozygotes at equilibrium between mutation and drift) ranged from 1.5 (*M. calcar*) to 26.83 (*P. parvivipara*) (Table 4.1). Overall, species with planktonic larvae had much lower theta H values (1.5-2.35), than either benthic egg mass layers (2.6-25.0) or livebearers (7.3-26.0). In species with benthic development and hermaphroditism there was a drastic increase in theta H, which suggests that lower levels of variation could be the result of loss of heterozygosity due to inbreeding.

*Planktotrophic Species* – Overall theta (H) for *M. mortenseni* and *P. pectinifera* were 1.52 and 1.53 respectively.

*Lecithotrophic Species* – Overall theta *(H)* for *M. calcar* and *C. pentagona* were 1.52 and 1.92 respectively. For *C. pentagona* standard error estimate on overall theta (H) was an order of magnitude higher than that of the other species with planktonic development.

*Benthic Egg Mass Laying* – Overall theta (H) for *P. exigua* was infinitely large. However, *P. exigua* shows theta H values that are consistently low in some regions (SH2 = 3.01, PS= 2.63), sometimes almost as low as those from the gonochoric *C. pentagona.* However, in South Australia *P. exigua,* significantly higher levels of theta H (PH = 25.668) suggest that populations in this region are experiencing different demographic conditions then those from New South Wales or Tasmania.

*Live Bearing Species* – Overall theta (H) values for all live bearing (*C. hystera, P. parvivipara, P. vivipara*) were infinitely large and had no real solution suggesting

that these species either had a high number of effective homozygotes with the same alleles or that there was not enough data to derive a numerical solution. For individual populations Theta H values ranged from 7.3 to 26.83 in *P. vivipara* and *P. parvivipara* while for all *C. hystera* populations theta (H) values were infinitely large.

## Genetic Variability Among Populations

### Bayesian Clustering Analysis STRUCTURE

The results of the STRUCTURE analysis suggests that, for the majority of species in this analysis, there were two significant population clusters for *M. calcar, P. exigua, P. parvivipara, P. pectinifera, C. hystera,* and *C. pentagona* (Figure 4.1). $\Delta$K values for k=2 were largest for all of these species, but the difference in $\Delta$K from 2 to 3 was not large for either *P. exigua* or *M. calcar,* suggesting that these species could divide into three clusters with greater sampling of individuals or loci (Figure 4.1 c and e).

*Planktotrophic Species* – The maximum $\Delta$K value for *M. mortenseni* was from k = 3, suggesting that three population clusters were most suitable for this species (Figure 4.1 b). In *M. mortenseni,* the result of three population clusters is somewhat equivocal. There are few individual assignments with high likelihood to any of the three population clusters in this analysis (Figure 4.1 b). *M. mortenseni* was the only species for which there were only 4 loci sampled. The small sample of loci could have prevented the program from reaching the true population grouping (Pritchard 2000). In planktotrophic *P. pectinifera,* populations

of individuals were assigned to two population clusters despite being sampled over a small range of about 10 km. A large proportion of individuals in populations FU and AB grouped into one cluster while most of the individuals form population OO grouped into a second cluster (Figure 4.1a).

*Lecithotrophic Species – M. calcar* populations were assigned to two clusters that roughly separated populations from Tasmania and South Australia in one cluster (except for Fortescue Bay Tasmania) and populations in New South Wales into another cluster. However, populations from Balmaural Bay (BM) in Sydney Harbour (New South Wales) cluster with populations from South Australia and Tasmania rather than with other populations in New South Wales (Figure 4.1 c). The lecithotrophic *C. pentagona* has two relatively well defined population clusters that are geographically peculiar in that the northernmost and southernmost (BB and AB1 respectively) populations sampled for this species are found in one cluster while the populations in between are found in the second cluster (Figure 1.2 for geographical orientation and Figure 4.1 d).

*Benthic Egg Mass Laying Species* – Similar to sympatric *M. calcar,* benthic egg laying *P. exigua* had two significant population clusters, one in South Australia and Tasmania (except for Fortescue Bay Tasmania) and the other in New South Wales. However, populations from Balmaural Bay (BM) in Sydney Harbour (New South Wales) cluster with populations from South Australia and Tasmania rather than with other populations in New South Wales (Figure 4.1 e).

*Live Bearing Species* – In live bearing *P. parvivipara* STRUCTURE separated populations into two clusters in concordance with the phylogeographic

hypotheses that I generated from the findings of previous research. One group consisted of populations from Ceduna (CED), Point Brown (PBR) and Granites (TGR), while the other group consisted of the lone Smooth Pools (SMP) population from Streaky Bay South Australia (Figure 4.1 f). The livebearer *P. vivipara* was the only species to show the highest likelihood of clusters at k=1 suggesting that there is very little subdivision among populations in this species. This species has equal frequencies of alleles in the same genotypic state (one common allele found in both homozygotes and heterozygotes, with a rare second allele that is found in multiple populations at similar low frequencies in heterozygotes) (Figure 4.1 g). Lastly, live bearing *C. hystera* showed population clustering that was not concordant with divergence between One Tree Island and mainland populations and instead shows population structure that is the result of a fixed allelic difference between OTI2, OTI4, and SY populations vs. those from OTI5 SB and OTI1 populations (Figure 4.1 h).

Population Pariwise $F_{st}$

Population pairwise $F_{st}$ values for individual loci ranged from -0.06 (*M. calcar,* PS vs. EN, C204a) to 1 in various population pairs and loci in *C. hystera, M. calcar, C. pentagona, P. exigua,* and *P. parvivipara.* Average pairwise $F_{st}$ values overall loci ranged from 0 (*P. parvivipara* TGR vs. PBR) to 1 (*C. hystera*) (Appendix 9). Species with benthic development and hermaphroditism often had higher pairwise $F_{st}$ values (overall $F_{st}$ ranging from 0.01 to 1.00) relative to species with planktonic larvae and separate sexes (overall $F_{st}$ ranging from -0.01 to 0.68). The seemingly large $F_{st}$ values (for microsatellites) derived from this

analysis are the result of the standardization method, which adjusts $F_{st}$ to a maximal value of one in order to make these values comparable among species with different underlying levels of diversity (Meirmans 2006).

*Planktotrophic Species* – In *P. pectinifera* pairwise $F_{st}$ ranged from -0.06 to 0.51 and two of three overall pairwise $F_{st}$ values were significantly different from zero (AS vs. OO $F_{st}$ = 0.19, and FU vs. OO $F_{st}$ = 0.51, P =0.001), despite the very small spatial scale of sampling in that species. Similarly for *M. mortenseni* pairwise $F_{st}$ ranged from -0.05-0.38 and two out of three population pairs had overall $F_{st}$ values that were significantly different from zero (KB vs. MB $F_{st}$ = 0.11, KB vs. WH $F_{st}$ = 0.07, P = 0.001).

*Lecithotrophic Species* – For *M. calcar* pairwise $F_{st}$ ranged from -0.01 to 1, and all but one overall population pairwise $F_{st}$ value was significantly different from zero (SH1 vs. DY $F_{st}$ = 0.01 P = 0.844). For *C. pentagona* $F_{st}$ ranged from 0.02 to 1.00 All *C. pentagona* populations had overall pairwise $F_{st}$ estimates that were significantly different from zero (P = 0.001 to 0.003).

*Benthic Egg Mass Laying Species* – In *P. exigua* pairwise $F_{st}$ ranged from 0.02 to 1.00 and all populations had overall pairwise $F_{st}$ estimates that were significantly different from zero (P = 0.001 to 0.003).

*Live Bearing Species* – In *P. parvivipara* pairwise $F_{st}$ ranged from 0.00 to 0.98 and three of six overall pairwise $F_{st}$ estimates differed significantly from zero (SMP vs. PBR $F_{st}$ =0.85, SMP vs. TGR $F_{st}$ = 0.79, SMP vs. CED $F_{st}$ = 0.86 P = 0.001). In *P. vivipara* pairwise $F_{st}$ ranged from -0.03 to 0.15 and one overall pairwise $F_{st}$ estimates differed significantly from zero (BH vs. EN, $F_{st}$ =0.15, P

=0.02). *C. hystera* had nine pairs of populations whose overall pairwise $F_{st}$ estimates differed significantly from zero. In those populations pairwise $F_{st}$ was equal to 1 and in the remainder of the population pairs for which $F_{st}$ did not differ significantly from zero $F_{st}$ was equal to 0.

Isolation By Distance

Benthic egg mass laying *P. exigua* was the only species that showed any evidence of IBD ($r^2 = 0.5$, P = 0.0007). This result depended mainly on the large geographic distance between the eastern samples and the South Australia populations: when South Australia populations were dropped from the analysis I found only a marginally significant matrix correlation between distance and differentiation ($r^2 = 0.32$, P =0.043)

**Population Genetic Differentiation: Analysis of Molecular Variance**

All AMOVA results can be found in table 4.3. Each AMOVA grouping was given a shorthand name for clarity in the AMOVA table. These shorthand names are accompanied by specific hypotheses and reference to their source (Table 4.2).

Within- and Among-Individual Variation

*Planktotrophic Species* – Overall within- and among- individual variation explained a significant proportion of the genetic variation for both *P. pectinifera* and *M. mortenseni.* Within-individual variation explained 72% and 71% of the population genetic variation respectively, and among–individual variation explained 21% and 24% of the total population genetic variation respectively.

*Lecithotrophic Species* – For *M. calcar* and *C. pentagona* within and among–individual variation explained a significant proportion of the variation and similarly high for both Species. Within-individual variation for *M. calcar* and *C. pentagona* was 67% and 49% respectively while among–individual variation explained 15% and 20% of the total genetic variation respectively.

*Benthic Egg Mass Laying Species* – For *P. exigua* within-individual variation explained 26% of the total genetic variation while among–individual variation explained only 5% of the total genetic variation, which although smaller than any of the planktonic species was significantly different from zero.

*Live Bearing Species – P. parvivipara, P. vivipara,* and *C. hystera* within- and among-individual variation was variable among species. In *P. parvivipara,* within-individual variation explained only 2% of the total genetic variation and among–individual variation explained a significant 27% of the total genetic variation. In *P. vivipara* within-individual variation explained 0% of the variation while among–individual variation explained 90% of the total genetic variation. In contrast within- and among-individual variation explained 0% of the total genetic variation for live bearing *C. hystera.*

Among-Population Variation

Similar to population pairwise Fst estimates, global estimates of $F_{st}$, which are calculated as the amount of total genetic variation that is accounted for among populations in an AMOVA were higher for species with benthic development and hermaphroditism than for gonochoric species with planktonic larvae.

*Planktotrophic Species* – In *M. mortenseni* 3% of the total genetic variation was explained by among-population genetic variation. In *P. pectinifera* 5% of the total genetic variation was explained by among population genetic variation. Although the proportions of total variance were small for both species they were statistically significant.

*Lecithotrophic Species* –In *M. calcar* 17% of the total genetic variation was explained among populations. In *C. pentagona* 30% of the total genetic variation was explained by among population genetic variation. Among population genetic variation was statistically significant for both lecithotrophic species.

*Benthic Egg Mass Laying Species – Parvulastra exigua* had an intermediate (between benthic live bearing and planktonic species) amount of total genetic variation explained among populations (67%), and this proportion was statistically significant.

*Live Bearing Species* – In two out of three species with live bearing development, among-population differentiation explained a large proportion of the variation (100% in *C. hystera,* 70% in *P. parvivipara).* The third live bearing species, *P. vivipara* showed surprisingly low among-population genetic variation, which explained only 9% of the total genetic variation. Although there was a large discrepancy among the proportions for live bearing species all results were statistically significant.

Among Group Variation: Predicted Phylogeographic Breaks

*Planktotrophic Species* – For *P. pectinifera* AMOVA revealed that the most significant proportion of the total genetic variation was explained by within-

individual variation (72%, P =0.001), consistent with the observed high diversity and high heterozygosity. When populations were separated according to the results from STRUCTURE, and populations from FU and AB where grouped separately from OO, among group variation explained a small and non-significant proportion of the total genetic variation (6%, P = 0.328).

All three AMOVA analyses for the planktotroph *M. mortenseni* suggested that within-individual genetic variation explained the largest proportion of the genetic variation in this group (~72% for all runs). The AMOVA analysis that grouped *M. mortenseni* populations into groups on the north and south ends of the north island of New Zealand in accordance with the findings of Spooner and Roy (2002) explained a non-significant proportion of the variation ($F_{ct}$ = 0, P = 0.38). The AMOVA that separated *M. mortenseni* populations into groups on the east and west sides of the north island of New Zealand (Waters and Roy 2004) explained a significant proportion of the variation ($F_{ct}$ = 0.025, P =0.025), although the relative percentage of the variation explained by this hierarchical level in the AMOVA was only 1.1%. Since the STRUCTURE results for this species were relatively inconclusive, and suggested high levels of admixture, an AMOVA based on the results of this analysis for this species was not run.

*Lecithotrophic Species* – In *M. calcar* within-individual variation explained the largest proportion of the total genetic variation in all AMOVA analyses (67%). This suggests (as in *M. mortenseni* and *P. pectinifera*) that populations have high diversity and individuals are likely to express high levels of heterozygosity and allelic diversity. Population groupings that separated *M. calcar* in concordance

with previous phylogeographic findings explained a relatively small proportion of the variation, some of which (EW = 11% and EWT = 6%) were significantly different from zero while others (TNT = -1%) were not. Although small relative to within-individual variation these results suggest that the phylogeographic hypothesis that explains the largest amount of the total genetic variation in *M. calcar* is that which groups populations in Tasmania and New South Wales together in a group separate from South Australia. *M. calcar* populations grouped according to the results from STRUCTURE (group 1 = EN (TAS), PS (TAS), ALA (SA), ALB (SA), BB (NSW), group 2 = ALL other NSW and TAS) explained the largest percentage of the variation ($F_{ct}$ = 0.27, 12% P = 0.001) of any of the AMOVA analyses where populations were grouped. Nevertheless, among group variation explained a small percentage of the variation relative to that explained by within-individual variation.

AMOVA analysis for lecithotrophic *C. pentagona* showed that within-individual variation (43-52%) explained the highest and most significant proportion of the total genetic variation. The only hypothesis of among group variation the explained a significant proportion of the variation was that of the results of STRUCTURE, which separates Bingal Bay (BB) with Arlie Beach 1 (AB1) into one group and Townsville (TV), Bowen (BO), Arlie Beach 2 (AB2) and Dingo Beach (DB) into the second group ($F_{ct}$ = 0.46, P = 0.00).

*Benthic Egg Mass Laying Species – AMOVA analyses of P. exigua* show that when populations were grouped according a priori hypothesis of population genetic structure the largest proportion of genetic variation was explained by

grouping populations on either side of Bass Straight (EW: group 1 = TAS and NSW, group 2 = SA) ($F_{ct}$= 0.64, 46% P = 0.04). The population grouping that explains the next greatest proportion of the total genetic variation is that which separates populations into three groups one in Tasmania (TAS) one in New South Wales and a third in South Australia (SA) ($F_{ct}$= 0.61, 45% P = 0.001). In contrast to the results of *M. calcar* AMOVA analysis based on STRUCTURE (which explained the highest proportion of the variation in *M. calcar*), the STRUCTURE group in *P. exigua* explained 28% of the total genetic variation ($F_{ct}$ = 0.401, P = 0.005), which although significant did not appear to be the best population grouping.

*Live Bearing Species* – In *P. parvivipara,* when populations were separated into two groups, that were concordant with the STRUCTURE results and an priori hypothesis based on previous phylogeographic findings (group 1 = Smooth Pools (SMP) inside Streaky Bay SA, group 2 = Ceduna (CED), Granites (TGR) and Point Brown (PBR) SA), the among group variation explained 85% of the total genetic variation. However, because of the relatively small amount of total population genetic variation in this species the results were not statistically significant ($F_{ct}$ = 0.857, P =0.256). For its congener *P. vivipara,* there was no apparent sign of population genetic differentiation and the low levels of variation prompted me to exclude this species from AMOVA with population grouping.

In live bearing *C. hystera* 100% of the genetic variation was explained among populations (P = 0.001). The AMOVA analysis that grouped populations on the mainland (SB, SY) and on the Great Barrier Reef (OTI1, OTI2, OTI4,

OTI5) explained 0% of the total genetic variation (P = 1). When among group differences were added to the AMOVA analysis that separated populations based on STRUCTURE results (group 1 = OTI1, SB, OTI5, group 2 = OTI2, OTI4, SY) the groupings explained 100% of the genetic variation but results were insignificant, probably because there was only one polymorphism that coincided with this population grouping (P = 0.105).

## .Discussion

My analysis suggests that overall both life history and, to a lesser extent, extrinsic ecological and historical factors contribute to population genetic structure in a group of closely related species. Overall, levels of within-population genetic variation were highly correlated with life history. Measures of heterozygosity, allelic richness and effective population size were all larger in species with a planktonic larval phase and separate sexes than in species with benthic development and live bearing. However, the distribution of genetic variation among populations differed among species. In gonochoric species with planktonic larval development the distribution of genetic variation among populations in most cases suggests low levels of genetic differentiation, however striking genetic heterogeneity exists in some parts of the geographic ranges of *M. calcar, P. pectinifera* and *C. pentagona.* Similarly, in hermaphroditic benthic developers there are overall high levels of genetic differentiation, however this differentiation is distributed among populations differently in species in different

regions of Australia. In one species, *P. vivipara,* genetic differentiation between populations is low relative to other live bearing species. These results suggest that patterns of gamete union functioning to structure genetic variation within populations are almost solely dependent on the larval dispersal modes and mating systems of asterinid species, but that genetic variation among populations may be dependent on a combination of intrinsic and extrinsic factors.

## Comparisons Among Species with Similar Life Histories

### *Planktotrophic Species*

*Meridiastra mortenseni* and *Patiria pectinifera*, have similar planktotrophic life histories but live in different environments and hemispheres. In both species levels of within-population genetic variation were high and both species have large effective population sizes. Similarly levels of pairwise population genetic differentiation, as well as overall $F_{st}$ estimates, were low relative to species with benthic development. This indicates that high levels of gene flow via planktonic larvae and/or large effective population sizes are acting to decrease the rate of genetic drift that may cause divergence of geographically distant populations. The STRUCTURE results and apparent high levels of admixture in *M. mortensoni,* and to some extent in *P. pectinifera,* suggests that there may be some signature of association among groups of alleles. The groupings found by STRUCTURE could be the result of a historical vicariant event and/or reduction of effective population size that has since been masked by more recent gene flow.

*M. mortenseni* and *P. pectinifera* show similar levels of within- among-population genetic variation despite being sampled on very different scales. One might expect a high degree of genetic similarity between *P. pectinifera* populations because populations were all sampled inside a small bay in Japan and the maximal distance between populations is on the order of 10 kilometres (OO and FU). Matsuoka and Asano (2003) found strikingly high levels of allozyme diversity in *P. pectinifera* in the same sampling region, suggesting that high gene flow and large effective population sizes could be acting to homogenize populations. However, significant population genetic differentiation was found at this small scale for one population (OO) relative to the other two populations (AS and FU). Alternatively, *M. mortenseni* populations, which were sampled up to 600 kilometres apart on opposite sides of the north island of New Zealand, have the potential to be highly isolated by diverging currents (west Auckland, east Auckland and East Cape see Figure 4.1). Similar to *P. pectinifera,* this species shows significant levels of population genetic differentiation for one population (KA) relative to two other populations (WA and MB). *M. mortenseni* and *P. pectinifera* exhibit relatively similar levels of genetic variation and heterozygosity within populations, which suggests that large effective population sizes, potentially associated with free-spawning and planktonic larvae, are contributing to patterns of variation. The moderate but significant degree of population subdivision could be associated with differences in the extrinsic factors experienced by these species, living in different regions and on differing geographic scales.

*Lecithotrophic Species*

Both *M. calcar and C. pentagona,* two species with lecithotrophic life histories, showed similar levels of allelic richness and heterozygosity within populations, as well as similar levels of among-population genetic variation despite being sampled on differing geographic scales. These species did however show differences in levels of regional differentiation in the AMOVA analysis; *M. calcar* exhibited regional differentiation whereas *C. pentagona* lacked any predictable pattern of regional differentiation. These differences may be explained the fact that *M. calcar* and *C. pentagona* populations have experienced historical factors associated with changing current patterns, the intrusion and retreat of glaciers and changing sea levels, differently in their different ranges. (Dawson 2005; Watters et al, 2008, Ayre et al. 2009). The factors and their long term effect on genetic structure of species could ultimately have led to regional differentiation (Tasmania and New South Wales vs. South Australia) that explained 15% of the overall variation in *M. calcar* and the seeming lack thereof in *C. pentagona*. Alternatively, the range of *C. pentagona* spans farther north through the Indo Pacific into southeast Asia, and an increase in geographic sampling of this species could reveal patterns of regional genetic structure similar to *M. calcar,* but not seen in this analysis.

*Benthic Live Bearing Species*

For the three benthic live bearing species *C. hystera, P. parvivipara* and *P. vivipara* levels of genetic variation were low relative to planktonic gonochoric species (see Appendix 4 for allelic richness). These results suggest that

inbreeding and philopatry in benthic live bearing species could be contributing to a higher rate of alleles drifting to extinction or fixation within populations. However, the distribution of this variation within and among populations varied greatly among species. For example although among population variation was high for both *P. parvivipara* and *C. hystera* different components of allelic and genotypic frequencies drove these patterns. In *C. hystera* high among population differentiation was driven by fixed differences among populations at locus B227 while in *P. parvivipara* high levels of among-population genetic variation were driven mostly by differences in genotype frequencies (differing heterozygosity) among populations.

**Comparisons Among Sympatric Species**

Lecithotrophic *Meridiastra calcar* and benthic egg mass laying *Parvulastra exigua,* species that have different mating systems and larval forms but a sympatric distribution, showed similar patterns of differentiation across their range. Both species show high levels of population genetic variation across the Bass Strait, a region that was once a land bridge linking Tasmania to mainland Australia, severing community continuity between marine species on either side of the land mass. Despite these species relative similarity in large-scale patterns of differentiation, levels of within-population genetic variation (allelic richness, effective population size, heterozygosity) differ between species. Heterozygosity in *M. calcar* was higher overall (0.03-0.9) than in *P. exigua* (0-0.6), and similarly allelic richness was lower for the benthic egg mass depositing species then for the planktonic lecithotroph. This pattern suggests that the hermaphroditic mating

system, and limited dispersal of *P. exigua* may be driving patterns of gamete union within populations, by increasing the chance of inbreeding among individuals.

A previous allozyme analysis by Hunt (1993) found that these species had differing levels of allelic and genotypic diversity within populations, which is concordant with my results in this region. Similarly, Hunt et al. (1993) found that patterns of among-population genetic differentiation differed in the two species. *P. exigua* had universally higher levels of population genetic subdivision relative to *M. calcar*, reflecting the limited dispersal ability *P. exigua*. Two subsequent studies have addressed the population genetic structure of *P. exigua* in regions spanning NSW, Tasmania, and either Victoria and/or South Australia. These studies were sampled on slightly differing scales and indeed show somewhat contrasting results of *P. exigua* phylogeographic structure. Colgan et al. (2005) suggested that contemporary eastward flow of the Leeuwin Current, low dispersal potential, exposure of the Bassmanian land bridge and the weakening of the EAC during the Pleistocene could be causing differentiation among regions for *P. exigua*. My results roughly match that of Colgan et al. however, I can not go further to explain the relative roles of these forces in structuring *P. exigua* populations.

Recently Ayre et al. (2009) conducted a study of sympatric species along the coast of New South Wales and Victoria, examining the importance of two biogeographic barriers to species in the region. Ninety Mile Beach is a stretch of soft sediment along the coastline of New South Wales that serves as a barrier to

many rocky shore species who cannot find suitable habitat in the region. A second barrier exists at Wilsons Promontory at the eastern edge of the Pleistocene Tasmanian land bridge. Biogeographic and phylogeographic differentiation is found around both of these barriers for many species of marine invertebrates (Dawson 2005, Hidas et al. 2007, and York et al 2009) and both oceanographic divergence of the EAC and Zeehan Currents as well as remnants of the effects of Pleistocene low sea levels stands are implicated in its cause. Although my analysis finds high levels of differentiation between Eastern and Western regions for both species, Ayre et al. did not find any differentiation of *P. exigua* in populations east and west of Wilsons Promontory. One likely explanation is differences in the geographic scale of population sampling in the two studies: my study included samples of P. exigua from farther west than those of Ayre et al. (2009), from which I was able to pick up patterns of differentiation that may have been absent from more localized sampling around Wilson's Promontory (Ayre et al. 2009; Waters and Roy and Colgan et al 2005).

One striking and surprisingly enigmatic result of this study is that *M. calcar* and *P. exigua* populations at Balmaural Beach NSW group with those from south Australia and Tasmania in both AMOVA and in the STRUCTURE outputs. However, pairwise *F*st estimates were only high between Balmaural beach and all other New South Wales populations for one locus in each of the species (B236 *M. calcar* and A110 in *P. exigua*), suggesting that selection on a particular trait linked to the loci in question might be driving this pattern. Another possible explanation is that Balmaural populations were secondarily introduced from

South Australian and/or Tasmanian populations. Balmaural Beach is in Sydney Harbour, a major shipping hub for the east coast of Australia. Ship ballast has been implicated in the introduction of several fish and invertebrate species in Sydney Harbour throughout the last 40 years (Holeck et al. 2004, Voisin et al. 2004). Individual *P. exigua* could have been transported by means of domestic shipping essentially homogenizing populations of Sydney Harbour with those from south Australia (Glasby et al. 2007; Lavioe et al. 1999).

**Comparisons Among Sister Taxa**

Live bearing *Parvulastra* species show similar levels of population genetic diversity measured as allelic richness and heterozygosity, however the distribution of this variation among populations differs among species. *Parvulastra vivipara* has much lower levels of among-population genetic differentiation then *P. parvivipara.* In *P. parvivipara* one locus (C212) with two alleles has a variable distribution of the second allele among populations. This heterogeneity leads to high variation in genotype and allele frequencies among populations and thus high levels of among-population genetic differentiation. Alternatively, at one locus (C219) in *P. vivipara* a second allele is found in multiple populations at similar frequencies (albeit low), leading to low levels of between population genetic differentiation due to similar allele and genotype frequencies among populations. These results suggest that while mating system and dispersal mode lead to similar levels of within-population genetic variation, differences in historical factors, ecology, and oceanography between the regions in which each species lives could be driving differences in patterns of among

population differentiation. Another possibility is that stochasticity of genetic drift through time, on a relatively small number of loci, is driving the differences between the two species. With additional sampling of loci among-population genetic variation may become qualitatively more similar, suggesting that lower heterozygosity in *P. vivipara* relative to *P. parvivipara* is merely an artefact of genetic drift happening slightly differently in these two species.

Lecithotrophic *C. pentagona* and live bearing *C. hystera* are sister species in which a change to benthic live bearing hermaphroditism from gonochorism with planktonic non-feeding larva has occurred. *C. pentagona* has higher levels of within-population genetic variation than *C. hystera*, which is expected for a gonochoric species with free-swimming larva. Similarly, among-population genetic differentiation is much higher in *C. hystera* than *C. pentagona.* Overall, these results suggest that that the gonochoric mating system and potential for dispersal via planktonic larva in *C. pentagona* and benthic live bearing in *C. hystera* may be driving these patterns. However, like the *Parvulastra* comparison above, because the two species are not sympatric I cannot rule out the possibility that differences in levels of within and between population genetic variation may be the result of extrinsic factors that differ between the ranges of these species.

Neither *Cryptasterina* species shows regional structuring in AMOVA analyses. In *C. hystera* fixed allelic differences at one locus showed no association with geographic distance or environmental variation. For example populations OTI 1 and OTI 4 were sampled at a distance of approximately 0.25 km from one another in high intertidal pools underneath coral rubble and yet were

fixed for different alleles at locus B227, while OTI1, OTI 5 and SB are separated by between 5 and 190 km but are fixed for a similar allele at B227. A similar pattern of allele distribution was seen in the hermaphroditic coral species *G. favulus* on the reef in and around One Tree Island (Miller and Ayre 2008). In *C. pentagona* STRUCTURE results and results of AMOVA analyses suggested that Airlie Beach 1 (AB1) and Bingal Bay (BB) populations formed a significant cluster. The isolation of the Bingal Bay population (but not the Airlie Beach population) agrees with predictions based on the current divergence near 15° S. If Bingal Bay populations were isolated by divergent current patterns, and either infrequent gene flow or convergence of allele frequencies united alleles then these populations could ultimately appear to be more similar.

**Role of Extrinsic vs. Intrinsic Factors**

The relationship between larval dispersal mode and population genetic structure is relatively consistent among species with similar life histories in different geographic regions, among species with differing life histories that live sympatrically and among sister taxa with similar or differing life histories. These results imply that gene flow via planktonic larvae and gonochoric vs. hermaphroditic mating systems are in large part responsible for driving these patterns. While it is highly probable that life history has a large role in structuring populations in this group, this analysis also demonstrates interactions with historical and/or contemporary extrinsic factors related to the geographic range of particular species' to determine population genetic structure.

One example of a strong contemporary extrinsic factor is oceanographic currents, which have the potential to greatly influence dispersal, especially in species with planktonic larvae. Along Australia's eastern coast strong influences of the East Australia Current (EAC) are implicated in shaping migration patterns of many marine species (Banks et al. 2010). The EAC begins from an inflow of the South Equatorial Current at 15° south latitude, and is seasonally variable, generally extending as far south as Southern Tasmania. Its flow pattern is strong and direct from 15°-33° and becomes less distinct with numerous outflows and eddies below 33° South (Figure 4.1). Although weaker than other Eastern Boundary currents such as the Gulf Stream, the EAC is an important component of nutrient upwelling and a cause of genetic differentiation among species (Ridgeway and Hill 2009). North of 33° south latitude, the strong flow of the EAC is thought to homogenize allozyme population structure of the intertidal snail *Bedeva hanleyi*, while south of 33° latitude offshore flow and strong eddies of the EAC causes decreased genetic diversity and higher levels of population genetic differentiation between populations south of this region (Hoskins 2000). The seasonally strong southward flow of the EAC that can extend as far south as Tasmania, may be responsible for generating similar patterns of population genetic variation among New South Wales and Tasmania populations in lecithotrophic *M. calcar* and benthic egg mass laying *P. exigua* despite differences in mating system and larval dispersal mode among these species. In both species New South Wales and Tasmania populations explain a high proportion of the total genetic variation when grouped together and compared to

the South Australia region, suggesting that a mechanism such as this strong ocean current is involved in making these regions less genetically isolated.

Species found on the south coast of Australia near the Great Australian Bight are subject to highly seasonal current patterns, and in some regions isolation due to eddies formed by the Leeuwin and Flinders Currents (Middleton and Bye 2007). In live bearing *Parvulastra parvivipara* measures of within-population genetic variation and a larger effective population size at Smooth Pools at Streaky Bay suggest that these populations maintain higher levels of variation than other populations. These results are consistent with previous phylogeographic findings in this region, which suggest that eddies formed by the Leeuwin current cause populations in the north east region of the Great Australian Bight to be genetically isolated (Fowler et al. 2000, Bilgman et al. 2007). Furthermore, *P. parvivipara* individuals are small in size (between 3 and 7 mm) and are subject to high rates of offshore advection, via wave action, and strong currents (personal comm. L. Roediger and M. Byrne), which could cause high rates of local population size reductions or even local extinction. If populations in Streaky Bay are indeed isolated from high wave exposure and strong currents then these populations may be able to maintain higher populations sizes for longer periods of time permitting new alleles to establish, spread throughout the population.

Although it is tempting to think of population genetic structure, or lack thereof, among asterinid species as differences in levels of gene flow influencing population genetic structure, evidence from recent studies by Marko et al. (2009),

McGovern et al. (2010) and Hart and Marko (2010) suggest that a large proportion of the genetic variation in contemporary populations is due to historical factors such as divergence time interacting with migration and effective population sizes. For example, the asterinid sea star *Patiria miniata* shares a similar genetic disjunction with several intertidal N.E. Pacific intertidal and subtidal invertebrate species. For many species similar vicariant events or current patterns have been used to explain similar population genetic patterns. However, McGovern et al. (2010) discovered that in many cases the interaction between divergence times, levels of migration and effective population sizes that ultimately direct patterns of genetic structure and that these factors act differently among species. Using the program IMa, McGovern et al. found that genetic divergence in *P. miniata* across Queen Charlotte Sound is quite ancient (~280,000 years) however the inference of large effective population sizes on either side of the disjunction along with a small but consistent level of migration is driving the population genetic structure that we see currently. Interestingly, the small but consistent level of migration found in *P. miniata* is consistent throughout its range (Baja California to SE Alaska), where in some regions (California) this species has low levels of population genetic differentiation. This evidence suggests that contemporary divergence of the California and Alaska currents near Queen Charlotte Sound does not appear to be limiting gene flow in this region relative to other regions such as California. Although gene flow in this species has less of a acute impact on population genetic differentiation, it does seem to be low and consistent among populations, suggesting that the

planktotrophic life history is contributing to genetic exchange between populations, but that temporal effects and changes in population size are more influential in creating large signals of genetic isolation among populations.

**Utility of Microsatellite Markers**

Marker choice is extremely important when setting out to answer questions about contemporary and historical population structure (Hellberg 2009). Population genetic studies that employ microsatellite genotypes to answer questions about the causes of differentiation are limited in their application to historical questions because the lack of a robust understanding of mutation rate or of the model of evolution of these markers makes it difficult to examine coalescent processes among populations within species (Balloux et al. 2000, Balloux and Moulin 2002, Hellberg 2009). There was a general lack of statistical conformation to normality of size frequency distributions in all of my microsatellite markers, which means that the assumption of a stepwise mutation model for most coalescent analyses would have been violated. Therefore, the omission of coalescent-based analyses of gene flow and effective population size from this analysis are warranted.

Microsatellites, however, are useful for their ability to detect fine scale genetic variation as well as to represent differentiation in many regions of the genome (Selkoe and Toonen 2006, Miller and Ayre 2008). Furthermore, the efficiency and low cost of microsatellite genotyping make them great markers for testing hypotheses of population genetic differentiation among multiple populations in multiple species.

Although I employ microsatellite markers for this analysis, I realize that these markers may not be able to reveal the processes underlying large-scale phylogeographic patterns, and that nuclear or mitochondrial sequence data (for which there are well know models of evolution) may be more suitable (Keever et al 2009). These data are being collected in collaboration with other researchers, and analysis for publication will include nuclear and mitochondrial sequence data as well as microsatellites. In this analysis I seek to detect signals of differentiation that are concordant with previous phylogeographic findings, for further tests using additional markers.

## .General Conclusions

This research contributes significantly to the body of research addressing relative influences of life history and extrinsic ecological factors on population genetic structure. Multi-species comparisons of this kind are rare in marine invertebrates (Teske et al. 2007 and Hickey et al. 2009; Lee and Boulding 2009) and while some studies have compared groups of closely related species (Hickey et al. 2009, Lee and Boulding 2009) most use communities that overlap in geographic range but that may not show similarities and differences in population genetic structure that arise in species with similar ancestry. Furthermore, for some of the species in this analysis this is the only study of population genetic structure to date. For example *P. vivipara,* a threatened Tasmanian sea star, has had little investigation of patterns of variation or differentiation. Similarly, the population genetics of marine invertebrates in South Australia and N.E. Australia

are understudied relative to North East Pacific communities. This research and sequence data collected in collaboration with this thesis will build on the relatively small body of literature on population genetics and phylogeography in this region.

This data is most significant in that it addresses both the intrinsic and to some extent the extrinsic causes of genetic differentiation Asterinidae. The information from this chapter will be used to inform my comparative analysis of the influence of life history on population genetic structure (See Chapter 7). More specifically, population genetic variation that was best explained by extrinsic factors in this analysis could provide a potential explanation for the residual variation associated with the correlation between life history and population genetic structure in the Asterinidae.

**Table 4.1 Effective population size measured at theta H (effective number of homozygotes) for each population in each species. Standard error estimates are reported for the total theta value for each species.**

| | Population | abrev | n | theta (H) | Std. error |
|---|---|---|---|---|---|
| ***P. miniata*** | | | | | |
| | Santa Barbara | SB | 33 | 1.522 | |
| | Bodega Bay | BB | 16 | 1.619 | |
| | Fort Bragg | FB | 20 | 1.595 | |
| | Bamfield | BAM | 48 | 1.526 | |
| | Tofino | NAM | 36 | 1.530 | |
| | Winter Harbour | WH | 50 | 1.594 | |
| | Tanu | TU | 96 | 1.578 | |
| | Louise Narrows | IN | 50 | 1.622 | |
| | Moresby Camp | QC | 15 | 1.500 | |
| | View Cove | VC | 15 | 1.560 | |
| | Dixon inlet | DI | 69 | 1.517 | |
| | Total | | 448 | 1.560 | 0.042 |
| ***P. pectinifera*** | | | | | |
| | Asmusi Bay | AS | 49 | 1.536 | |
| | Futagojima | FU | 49 | 1.529 | |
| | Ooura | OO | 52 | 1.509 | |
| | Total | | 150 | 1.525 | 0.011 |
| ***M. mortensoni*** | | | | | |
| | Karaka | KA | 45 | 1.526 | |
| | Mission Bay | MB | 18 | 1.554 | |
| | Whangarei | WH | 32 | 1.511 | |
| | Total | | 95 | 1.531 | 0.018 |
| ***M. calcar*** | | | | | |
| | Shell Harbour 2 | SH2 | 46 | 1.512 | |
| | Little Bay | LB | 40 | 1.548 | |
| | Eaglehawk Neck | EN | 27 | 1.526 | |
| | Balmaural Beach | BM | 32 | 1.547 | |
| | Clovelley | CL | 46 | 1.502 | |
| | Deewhy | DY | 38 | 1.501 | |
| | Primrose Sands | PS | 32 | 1.500 | |
| | Allison Beach | AB | 31 | 1.500 | |
| | Fortescue Bay | FB | 29 | 1.523 | |
| | Gordons Bay | GB | 43 | 1.507 | |
| | Port Stevens | PS1 | 46 | 1.500 | |
| | Adelade a | ALA | 29 | 1.559 | |
| | Adelade b | ALB | 28 | 1.546 | |
| | Total | | 467 | 1.521 | 0.021 |
| ***C. hystera*** | | | | | |
| | One Tree Island 2 | OTI2 | 32 | inf | |
| | One Tree Island 1 | OTI1 | 40 | inf | |
| | One Tree Island 4 | OTI4 | 29 | inf | |
| | Statute Bay | SB | 29 | inf | |
| | South Yeppoon | SY | 20 | inf | |
| | One Tree Island 52t | OTI5 | 43 | inf | |
| | Total | | 193 | inf | 0.000 |

| | Population | abrev | n | theta (H) | s.d. |
|---|---|---|---|---|---|
| ***C. Pentagona*** | | | | | |
| | Arlie beach 2 | AB2 | 32 | 1.672 | |
| | Arlie Beach 1 | AB1 | 32 | 2.019 | |
| | Bingal Bay | BB | 31 | 2.356 | |
| | Bowen | BO | 32 | 1.906 | |
| | Townsville | TV | 40 | 1.606 | |
| | Dingo Beach | DB | 30 | 2.003 | |
| | Total | | 197 | 1.927 | 0.247 |
| ***P. exigua*** | | | | | |
| | Primrose Sands | PS | 41 | 2.639 | |
| | Shell Harbour 1 | SH1 | 45 | 3.211 | |
| | Clovelley | CL | 40 | 6.117 | |
| | Eaglehawk Neck | EN | 28 | 4.749 | |
| | Deewhy Beach | DY | 40 | 3.371 | |
| | Little Bay | LB | 48 | 6.931 | |
| | Port Stevens 1 | PS1 | 29 | inf | |
| | Tikera | TK | 15 | 13.445 | |
| | Shell Harbour 2 | SH2 | 32 | 3.009 | |
| | Balmaural Beach | BM | 43 | 4.739 | |
| | Port Hughes | PH | 20 | 25.668 | |
| | Total | | 381 | inf | 0.000 |
| ***P. vivipara*** | | | | | |
| | Blowhole | BH | 28 | inf | |
| | Fortescue Bay | FB | 37 | 7.313 | |
| | Eaglehawk Neck | EN | 19 | 12.943 | |
| | Total | | 84 | inf | 0.000 |
| ***P. parvivipara*** | | | | | |
| | Ceduna | CED | 20 | | |
| | Point Brown | PBR | 51 | 26.838 | |
| | The Granites | TGR | 50 | 14.081 | |
| | Smooth Pool | SMP | 36 | 9.471 | |
| | Total | | 157 | inf | 0.000 |

**Table 4.2 Region specific predictions of among group population genetic variation in asterinid sea stars. The abbreviations for each prediction are used in the text and in table 4.3. The Phylogeographic break denotes the regional grouping of populations for each prediction. Groups A, B and C are arbitrary ways to denote different groupings for each prediction. Source of prediction labelled STRUCTURE is derived from the results of the STRUCTURE analysis for each species , Groupings are separated by region and by species.**

| Title | Phylogeographic Break | Species | Source of Prediction |
|---|---|---|---|
| **Japan** | | ***Patiria pectinifera*** | |
| STR1 | group A - Futagojima, Anasmusi Bay Japan<br>group B - Ooura Japan | | STRUCTURE |
| **New Zealand** | | ***Meridiastra mortenseni*** | |
| MORT1 | group A - the north end of the North Island of New zealand<br>group B - the south end of the south island of New Zealand | | Spooner and Roy 2002 |
| MORT2 | group A - the esat side of the North Island of New Zealand<br>group B - the west side of the North Island of New Zealand | | Waters and Roy 2004 |
| **New South Wales, Tasmania, South Australia** | | ***Meridiastra calcar***<br>***Parvulastra exigua*** | |
| TNT | group A - populations from Tasmania<br>group B - populations from New South Wales and South Australia | | Fraser et al. 2009 |
| EW | group A - Tasmania and New South Wales<br>group B - populations from South Australia | | Waters 2003, Dawson 2005, Waters et al. 2006, York et al. 2008 |
| EWT | group A - populations from Tasmania<br>group B - populations from South Australia<br>group C - populations from New South Wales | | Waters 2003, Dawson 2005, Waters et al. 2006, York et al. 2008Fraser et al. 2009 |
| STR2 | group A - Eaglehawk Neck Primrose Sands South Australia and Bingal Bay New South Wales<br>group B - all other populations sampled in New South Wales and Tasmania | | STRUCTURE |

| **Great Barrier Reef** | | ***Cryptasterina hystera*** | |
|---|---|---|---|
| GBRNOGBR | group A - populations on the Great Barrier Reef OTI1, OTI2, OTI4, OTI5<br>group B - populations on Mainland Australia in Queensland including Statute Bay and South Yeppoon | | Doherty et al. 1994, Doherty et al. 1995 |
| STR3 | group A - populations on the Great Barrier Reef OTI1 and OTI5 and in Queensland Australia, Statute Bay<br>group B - populations on the Great Barrier Reef OTI2 and OTI4 and Queensland populations South Yeppoon | | STRUCTURE |
| **Queensland** | | ***Cryptasterina pentagona*** | |
| TVBB | group A - Townsville and Bingal Bay<br>group B - Dingo Beach, Arlie Beach1 and Arlie Beach 2 and Bowen | | Haig et al. 2010 |
| BBB | group A - Bingal Bay<br>group B - Townsville, Dingo Beach, Arlie Beach1, Arlie Beach2, and Bowen | | Haig et al. 2010 |
| STR4 | group A - Bingal Bay and Arlie Beach 1<br>group B - Townsville, Dingo Beach, Arlie Beach 2, Bowen | | STRUCTURE |
| **South Australia** | | ***Parvulastra parvivipara*** | |
| SMP | group A - Smooth Pools inside Streaky Bay South Australia<br>group B - Ceduna, The Granites, and Point Brown | | Fowler et al. 2000, Bilgman et al. 2007 |

**Table 4.3 AMOVA Table for 8 asterinid species from population genetic analysis. F' represents a measure of among population variation standardized to 1. Predictions of among group variation for each species are found in the column labelled predictions. Specific details on each prediction of among group variation can be found in Table 4.2.**

| Source of Variation | %var | F-value | p-value | F '-value | Prediction |
|---|---|---|---|---|---|
| ***P. pectinifera*** | | | | | |
| Within Individual | 0.727 | 0.273 | -- | | |
| Among Individual | 0.219 | 0.231 | 0.001 | | |
| Among Population | 0.054 | 0.054 | 0.001 | 0.098 | |
| Within individual | 0.713 | 0.287 | -- | | |
| Among Individual | 0.214 | 0.231 | 0 | | |
| Among Population | 0.01 | 0.011 | 0.032 | 0.02 | |
| Among Group | 0.063 | 0.063 | 0.328 | 0.114 | STR1 |
| ***M. mortenseni*** | | | | | |
| Within Individual | 0.714 | 0.286 | -- | -- | |
| Among Individual | 0.247 | 0.257 | 0.001 | -- | |
| Among Population | 0.038 | 0.038 | 0.001 | 0.087 | |
| Within Individual | 0.715 | 0.285 | -- | -- | |
| Among Individual | 0.248 | 0.257 | 0.001 | -- | |
| Among Population | 0.039 | 0.039 | 0.002 | 0.088 | |
| Among Group | -0.002 | -0.002 | 0.348 | -0.005 | MORT1 |
| Within Individual | 0.713 | 0.287 | -- | -- | |
| Among Individual | 0.247 | 0.257 | 0.001 | -- | |
| Among Population | 0.03 | 0.03 | 0.013 | 0.069 | |
| Among Group | 0.011 | 0.011 | 0.001 | 0.025 | MORT2 |
| ***M. calcar*** | | | | | |
| Within Individual | 0.672 | 0.328 | -- | -- | |
| Among Individual | 0.154 | 0.186 | 0.001 | -- | |
| Among Population | 0.174 | 0.174 | 0.001 | 0.333 | |
| Within Individual | 0.678 | 0.322 | -- | -- | |
| Among Individual | 0.155 | 0.186 | 0.001 | -- | |
| Among Population | 0.18 | 0.178 | 0.001 | 0.339 | |
| Among Group | -0.014 | -0.014 | 0.291 | -0.034 | TNT |
| Within Individual | 0.653 | 0.347 | -- | -- | |
| Among Individual | 0.149 | 0.186 | 0.001 | -- | |
| Among Population | 0.144 | 0.152 | 0.001 | 0.29 | |
| Among Group | 0.115 | 0.115 | 0.001 | 0.252 | EW |
| Within Individual | 0.652 | 0.348 | -- | -- | |
| Among Individual | 0.149 | 0.186 | 0.001 | -- | |
| Among Population | 0.137 | 0.146 | 0.001 | 0.279 | |
| Among Group | 0.062 | 0.062 | 0.009 | 0.14 | EWT |
| Within individual | 0.627 | 0.373 | | -- | |
| Among Individual | 0.143 | 0.186 | 0 | -- | |
| Among Populatio | 0.102 | 0.117 | 0 | 0.224 | |
| Among Group | 0.128 | 0.128 | 0.001 | 0.276 | STR2 |
| ***C. hystera*** | | | | | |
| Within Individual | 0 | 1 | -- | -- | |
| Among Individual | 0 | nan | 0.001 | -- | |
| Among Population | 1 | 1 | 0.001 | 1 | |
| Within Individual | 0 | 1 | -- | -- | |
| Among Individual | 0 | nan | 0.001 | -- | |
| Among Population | 1 | 1 | 0.001 | 1 | |
| Among Group | -0.474 | -0.474 | 1 | -0.58 | GBRNOGBR |
| Within individual | 0 | 1 | -- | -- | |
| Among Individual | 0 | -- | -- | -- | |
| Among Populatio | 0 | -- | -- | -- | |
| Among Group | 1 | 1 | 0.105 | 1 | STR3 |
| ***C. pentagona*** | | | | | |
| Within Individual | 0.49 | 0.51 | -- | -- | |
| Among Individual | 0.206 | 0.295 | 0 | -- | |

| | | | | | |
|---|---|---|---|---|---|
| Among Population | 0.304 | 0.304 | 0 | 0.43 | |
| Within Individual | 0.523 | 0.477 | -- | -- | |
| Among Individual | 0.219 | 0.295 | 0 | -- | |
| Among Population | 0.412 | 0.357 | 0 | 0.503 | |
| Among Group | -0.155 | -0.155 | 0.657 | -0.287 | TVBB |
| Within Individual | 0.492 | 0.508 | -- | -- | |
| Among Individual | 0.206 | 0.295 | 0 | -- | |
| Among Population | 0.306 | 0.305 | 0 | 0.434 | |
| Among Group | -0.003 | -0.003 | 0.5 | -0.005 | BBB |
| Within Individual | 0.431 | 0.569 | -- | -- | |
| Among Individual | 0.181 | 0.295 | 0 | -- | |
| Among Population | 0.082 | 0.119 | 0 | 0.168 | |
| Among Group | 0.306 | 0.306 | 0 | 0.459 | STR4 |
| ***P. exigua*** | | | | | |
| Within Individual | 0.263 | 0.737 | -- | -- | |
| Among Individual | 0.058 | 0.18 | 0.001 | -- | |
| Among Population | 0.679 | 0.679 | 0.001 | 0.764 | |
| Within Individual | 0.21 | 0.79 | -- | -- | |
| Among Individual | 0.046 | 0.18 | 0.001 | -- | |
| Among Population | 0.445 | 0.634 | 0.001 | 0.713 | |
| Among Group | 0.299 | 0.299 | 0.051 | 0.441 | TNT |
| Within Individual | 0.225 | 0.775 | -- | -- | |
| Among Individual | 0.049 | 0.18 | 0.001 | -- | |
| Among Population | 0.467 | 0.63 | 0.001 | 0.708 | |
| Among Group | 0.46 | 0.46 | 0.041 | 0.644 | EW |
| Within Individual | 0.2 | 0.8 | -- | -- | |
| Among Individual | 0.044 | 0.18 | 0.001 | -- | |
| Among Population | 0.298 | 0.55 | 0.001 | 0.619 | |
| Among Group | 0.457 | 0.457 | 0.001 | 0.616 | EWT |
| Within individual | 0.228 | 0.772 | | | |
| Among Individual | 0.05 | 0.18 | 0 | | |
| Among Populatio | 0.44 | 0.613 | 0 | 0.689 | |
| Among Group | 0.281 | 0.281 | 0.005 | 0.401 | STR2 |
| ***P. parvivipara*** | | | | | |
| Within Individual | 0.021 | 0.979 | -- | -- | |
| Among Individual | 0.271 | 0.929 | 0.001 | -- | |
| Among Population | 0.708 | 0.708 | 0.001 | 0.73 | |
| Within Individual | 0.012 | 0.988 | -- | -- | |
| Among Individual | 0.156 | 0.929 | 0 | -- | |
| Among Population | 0.001 | 0.008 | 0.177 | 0.008 | |
| Among Group | 0.831 | 0.831 | 0.256 | 0.857 | SMP |
| ***P. vivipara*** | | | | | |
| Within Individual | 0 | 1 | -- | -- | |
| Among Individual | 0.902 | 1 | 0.001 | -- | |
| Among Population | 0.098 | 0.098 | 0.033 | 0.102 | |

**Figure 4.1 Major sea surface currents in Southern and Eastern Australia and New Zealand.**

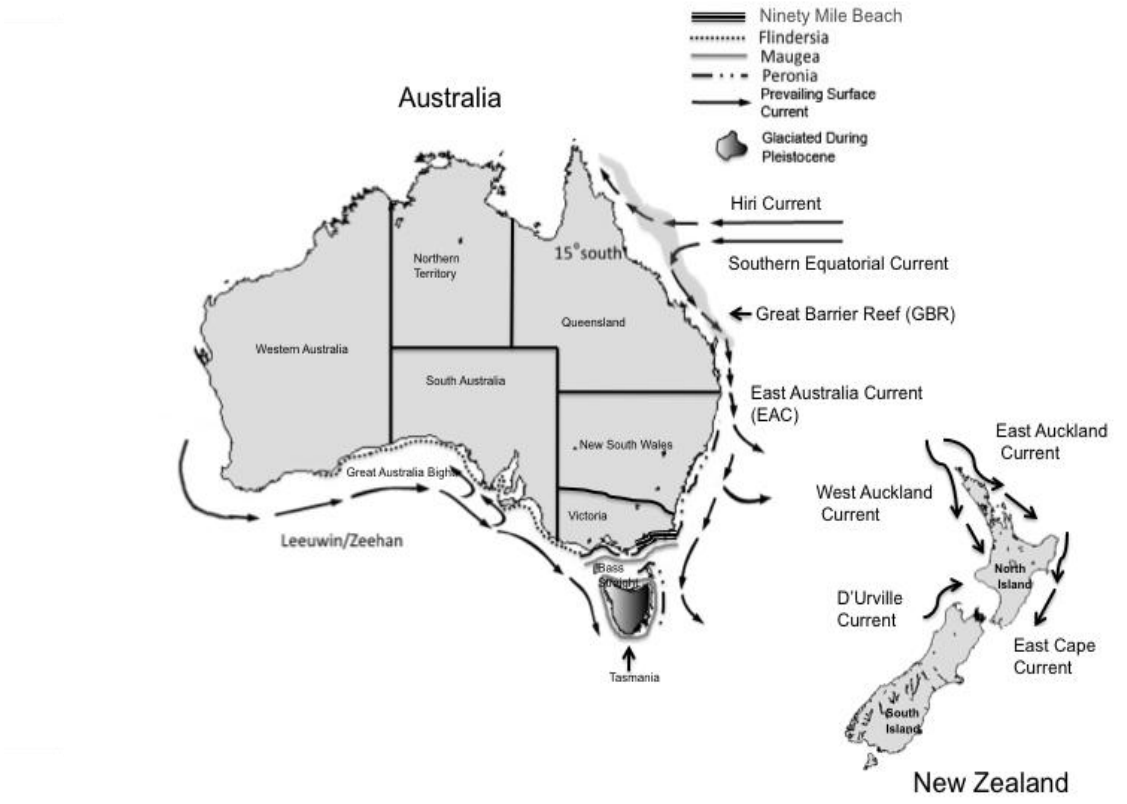
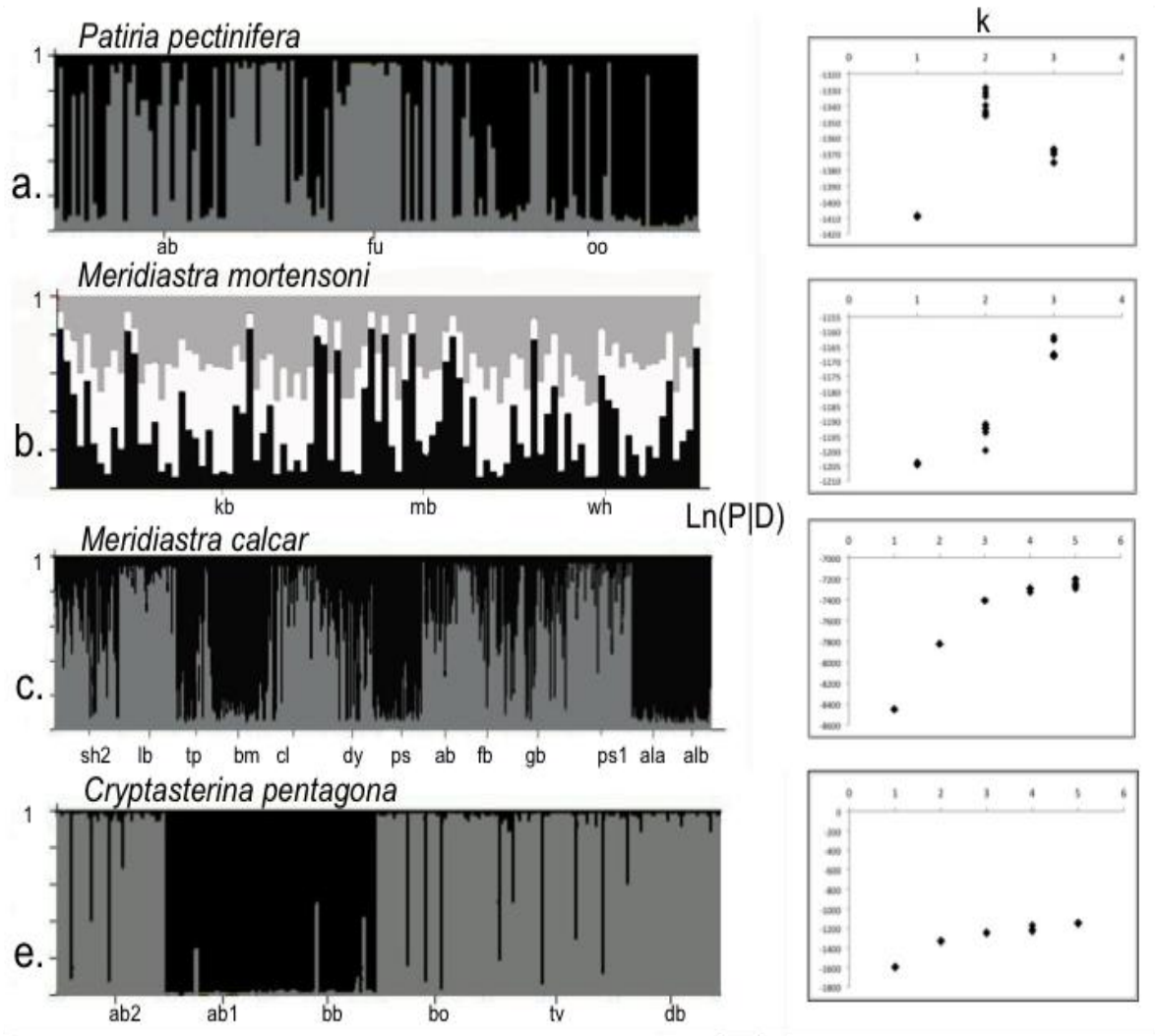
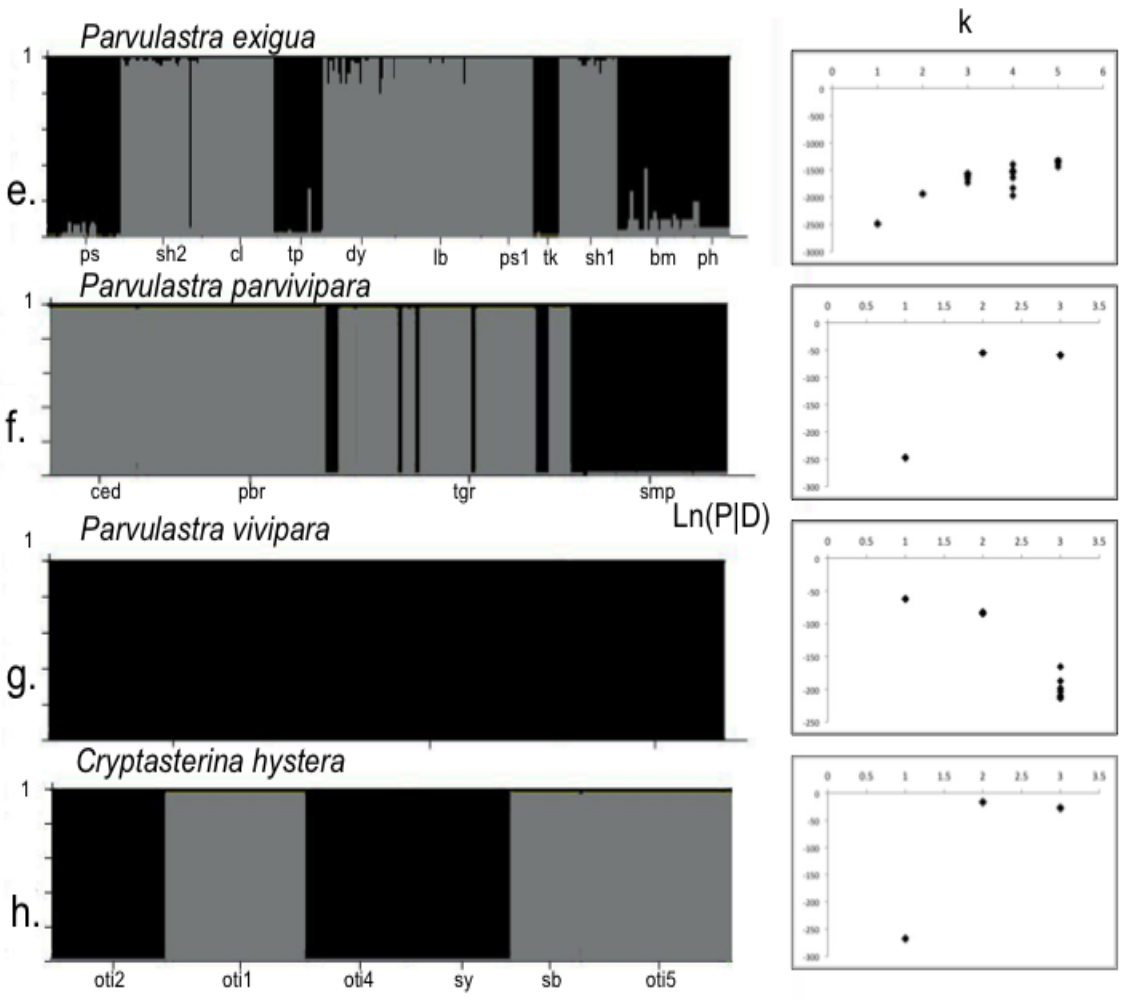


**Figure 4.2 Results of the STRUCTURE analysis for each species of asterinid. Graphs to the right represent delta K estimates of Evano et al. 2005. Population clusters with the highest change in the ML value of K are the most likely. For each species the number of colours represents the most likely number of population clusters. Populations are labelled at the bottom of each graph and abbreviations can be found in Appendix 1.**




k
Parvulastra exigua
1
e.
ps
sh2
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dy
lb
ps1
tk
sh1
bm
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Parvulastra parvivipara
1
f.
ced
pbr
tgr
smp
Ln(P|D)
Parvulastra vivipara
1
g.
Cryptasterina hystera
1
h.
oti2
oti1
oti4
sy
sb
oti5

# Chapter 5 **NOWHERE TO RUN TO BABY: ANALYSIS OF MATING SYSTEM AND BROODING ALLOMETRY IN LIVE BEARING *PARVULASTRA* SPECIES**

## .Introduction

Differences in the frequency of mating between close relatives can influence population genetic variation by changing patterns of gamete union within individuals and among conspecifics (Clay 1982, Loveless and Hamrick 1984, Knowlton and Jackson 1993, Grosberg and Cunningham 2001, Karl 2008). Differences in patterns of gamete union alter genotype frequencies and change the distribution of alleles throughout a population (Wright 1965). In gonochoric species (with separate sexes), mating system variation mainly depends on the spatial distribution of close and distant relatives, the frequency of mating encounters between relatives, and behavioural and cellular aspects of mate choice (Jarne 1993). In simultaneous hermaphrodites, an additional mating system variable is the frequency of individuals that self-fertilize (an extreme form of mating between closely related male and female germ lines). Mixed mating systems, where both self-fertilization and outcrossing occur within the same clutch of eggs, occur among many plant and a few animal species (Uyenoyama 1986, Goodwillie et al. 2005, Jarne and Auld 2007).

In the previous chapter I documented extraordinary loss of heterozygosity, with important consequences for population differentiation, in three simultaneous

hermaphrodite asterinid species (*Cryptasterina hystera, Parvulastra vivipara, P. parvivipara*). Because these species also lack planktonic larval dispersal, both outcrossing between close relatives (via the local recruitment of siblings near each other and near their parents) and selfing (via the simultaneous development of eggs and sperm in single individuals, with internal fertilization of eggs) could contribute to loss of heterozygosity and strong genetic drift.

Here I analyze genetic variation within broods of two live bearing species *Parvulastra vivipara* and *Parvulastra parvivipara*. I use microsatellite markers to genotype parents and offspring from multiple broods to determine first whether there is evidence that offspring are the product of sexual reproduction (rather than some unexpected form of parthenogenesis), and secondly whether there is evidence of outcrossing or multiple paternity within clutches. I also take advantage of the availability of large numbers of broods in one species in order to test a hypothesis regarding the association between small adult body size and the evolution of brooded modes of reproduction.

The small egg sizes of live-bearing *Parvulastra* species relative to other asterinid species with non-feeding larval forms is likely associated with intragonadal cannibalism of siblings (Byrne 2006). Offspring grow from small eggs through feeding on brood-mates (analogous to growth from small egg size by consumption of phytoplankton by larvae of planktotrophic species). The success of this strategy for brood provisioning may be constrained by the size and resources of the adult parent (Strathmann and Strathmann 1984). In particular, allometric variation in adult size, fecundity, and brooding capability

may disproportionately affect the brooding success of large compared to small adults such that large adults (in spite of their higher fecundity) suffer a disproportionate loss of brooded larvae compared to smaller adults. This allometric constraint has been suggested as an explanation for the coevolution of small adult body size and brood protection in various marine invertebrate taxa. However, evidence in support of this allometric hypothesis is limited (Kabat 1985, Hess 1993, Sewell 1994, Fernandez et al. 2006). In particular, Byrne (1996a) found no evidence of a scaling constraint between clutch size and parent size in either of these *Parvulastra* species.

Here I examine brood characteristics for evidence of such an allometric constraint associated with brood cannibalism. I measure parent size (in one species), plus clutch size and offspring size (in both species) in order to determine how clutch size decreases as offspring grow. For example, if sibling brood cannibalism is responsible for the decrease in clutch size then smaller broods should consist of large offspring (Byrne 1996a).

Finally, I revisit the relationship between adult diameter and clutch size found in Byrne (1996), using my brood measurements to test the associations between adult size, clutch size, and average offspring size. The relationship between adult size and various stages of brooding can shed light on how adult size might limit maternal brooding capacity. I used the difference in the relationship between clutch size and offspring size among large versus small females to test the prediction that large females suffer disproportionately greater losses of brooded offspring (possibly due to cannibalism) (Strathmann and

Strathmann 1982; Strathmann et al. 1984, Ernissee 1988, Hess 1993, Byrne 1996a).

**Study System**

*Parvulastra parvivipara* is endemic to the Eyre Peninsula in South Australia, reproduces year round, and is the smallest asterinid with maximum adult size of 3 to 7 millimetres (Keough and Dartnall 1978). Individuals are distributed at high densities in high intertidal sandstone pools and under rocks (Byrne 1996a, Roediger and Bolton 2008). *P. parvivipara* is a simultaneous hermaphrodite capable of self-fertilization and possibly asexual reproduction. Individual gonads include both ovary and testis tissues, with a female-biased sex allocation of about 6:1. Eggs are small relative to other asterinid species with non-feeding development (Byrne 1996b). Fertilization occurs in the gonads and offspring are brooded internally. Adult *P. parvivipara* brood offspring in interambulacral spaces and juveniles emerge from a distended gonophore. Some degree of maternal provisioning of nutrients as well as a significant amount of sibling cannibalism has been reported (Byrne 1996a). Adults are capable of self-fertilization and produce much more sperm than necessary for this function alone, and this over-production of sperm may suggest the potential for outcrossing (Byrne 1996). The relationships among brood mates are unknown.

*Parvulastra vivipara* is endemic to the east coast of Tasmania. Maximum adult size of 15 millimetres is slightly larger than *P. parvivipara* (Dartnall 1969). Individuals are distributed in the high intertidal in small aggregations under rocks

and in crevices. Most gonads are ovaries, but ovotestes (like those of *P. parvivipara*) as well as pure testes are found (Byrne 1996a). Eggs of this species are small relative to other asterinid species with non-feeding larvae. Individuals reproduce year-round with a peak from November to January. Isolated individuals in the lab can live for up to 8 years and are capable of reproduction, presumably by self-fertilization or possibly by asexual reproduction (Prestedge 1998). However, the degree to which outcrossing occurs in this species is unknown. Some degree of maternal provisioning of developing embryos as well as sibling cannibalism has been documented in this species (Byrne 1996a).

## .Methods

Dissected *Parvulastra vivipara* broods were provided as frozen samples by Maria Byrne at the University of Sydney (Australia). My analysis was limited to previously collected broods because this species is threatened by coastal development and new collections are prohibited. Samples were originally collected in 1991 from Eaglehawk Neck on the east coast of Tasmania (N=19), and from a nearby population at the Blowhole (N=1). Unfortunately, only brooded juveniles and a small sample of parental tissue was available, and thus information on the attributes of the parent such as size are missing from this analysis. In the laboratory at Simon Fraser University I counted individual juveniles from each brood and measured juvenile diameter using a calibrated ocular micrometer on a dissecting microscope.

*Parvulastra parvivipara* adults were collected from The Granites in October 2005, and from the Smooth Pools population in October 2005 and October 2009 (Figure 1.2). I measured the diameter of 100 adults in the lab at Simon Fraser University using a calibrated ocular micrometer. Measurements were taken along the widest inter-radial section of the individual's body. I dissected adults by cutting and lifting off a circular section of the aboral surface of each individual (Figure 5.1). Removal of the aboral section revealed the brooded offspring in each interambulacrum. I recorded the clutch size for each brood. Broodmates were transferred with sterilized forceps to a petri dish and measured on a dissecting microscope. Offspring were then placed in individual 1.5ml micro-centrifuge tubes for DNA extraction (n=378).

I used a standard CTAB DNA extraction protocol for parents and offspring as in Chapters 2, 3 and 4. I genotyped *P. vivipara* parents and offspring at the single locus that was polymorphic within population samples of that species *(*C219) plus two other loci, one that cross-amplified in all asterinid species (B202, to examine this locus for inheritance and the potential for null alleles) and one that was polymorphic in *P. parvivipara* (C212). *P. parvivipara* broods were genotyped at two loci known to be polymorphic in population samples of that species (C212 and A110). PCR conditions and genotyping methods were the same as those in Chapters 2, 3 and 4. I looked for Mendelian segregation of maternal alleles among offspring of a heterozygous parent as evidence of probable sexual reproduction (by either selfing or outcrossing). I looked for non-

maternal alleles in one or more offspring from a single brood as evidence of outcrossing.

For each species I examined the relationship between clutch size and average offspring size in R. (Development Core Team 2005, www.R-project.org). I used a standard linear model to calculate the relationship between clutch size and offspring size using the 'lm' function.

Previous studies (Kabat 1985, Hess 1993, Sewell 1994 Fernandez et al. 2006) have examined the relationship between small adult size and brooding in marine animals (Strathmann and Strathmann 1982) by testing inter- and intra-specific associations between parent size, clutch size and offspring survival or growth. I use data on parent size, offspring size and clutch size to investigate the potential for a fitness advantage in smaller *P. parvivipara* parents relative to large parents.

For *P. parvivipara* data points in the linear model, the corresponding parent size information was binned into four parent size classes with similar numbers of broods in each bin (bin 1 = 3.0 – 4.8 mm, bin 2 = 5.0 – 5.9 mm, bin 3 = 6.0 – 6.8 mm, bin 4 = 7.0 – 9.0 mm) and I used ANCOVA to specifically address the association between clutch size and offspring size for each of the four bins and among the bins. Furthermore, for both *P. vivipara* and *P. parvivipara* I calculated the variance in offspring size per clutch. I examined the relationship between the variance in offspring size and clutch size using a linear regression. For *P. parvivipara* I also measured the partitioning of variance in

clutch size, average offspring size and adult size among populations using a two way ANOVA.

I plotted residual variation in all linear regression analyses and tested residual values for normality using a Shapiro-Wilk normality test, and of the three models I tested none of the residual variation could reject the null hypothesis of normality (offspring size vs. clutch size P = 0.18, parent size vs. offspring size P = 0.20, and parent size vs. clutch size P = 0.4). I also plotted residual variation against fitted values for each of the linear models and there was no clear trend between residuals and fitted values for any of the three models (Zuur et al 2009).

## .Results

### *P. vivipara* Brood Characterization

In total 20 broods from *P. vivipara* were examined. In three broods I was unable to collect full genotypes at all three loci for all offspring and 1-3 genotypes are missing from 1-4 individuals. Clutches ranged in size from 1 to 6 offspring and offspring size ranged from 0.8 mm to 4 mm.

In total, I genotyped 20 parents and 74 offspring. All 20 brood parents were homozygous at all three loci used in this analysis. Similarly, all offspring were homozygous for the maternal allele.

Clutch size was not correlated to mean offspring size ($r^2 = 0.04$, P = 0.40) (Figure 5.2a). This is a surprising result in light of the potential for cannibalism among broodmates to reduce clutch size while simultaneously increasing the size

of the remaining offspring. Similarly there was no significant relationship between variance in offspring size and clutch size in *P. vivipara* ($R^2 = 0$, $P = 0.781$), suggesting variance in size among brood-mates does not decrease as broods become smaller.

### *P. parvivipara* Brood Characterization

Altogether I measured 378 offspring from 100 parents from two populations (50 broods from The Granites, 35 from the 2005 Smooth Pools collection, and 13 from the 2009 Smooth Pools collection). I was able to fully genotype 42 *P. parvivipara* clutches and their parents at two polymorphic loci (24 from The Granites, 15 from the 2005 Smooth Pools collection, and 3 from the 2009 Smooth Pools collection). The remaining broods had either incomplete sets of genotypes collected due to faulty DNA extractions (~30), or were not genotyped. For the genetic analysis I focus only on broods for which full genotypes were collected. In one brood included in my genotyping analysis (smp32) I was unable to genotype one individual at each locus.

Clutches ranged in size from 1 to 11 with offspring sizes ranging from 0.2 mm to 1.7 mm. Parent size ranged from 3mm to 7mm.

I genotyped 42 parents and 181 offspring at two loci. One parent was heterozygous at A110 (for alleles of 228 and 232 bp), and there were no heterozygous parents at C212. In all cases the frequency of the second alleles at each polymorphic locus in *P. parvivipara* was low and as such, it is not surprising that I found so few heterozygous parents in my brood analysis. For the brood associated with the heterozygous parent, all four siblings were

homozygous for one of the two maternal alleles (228/228 for smp36-1, smp36-2, smp36-4; and 232/232 for smp36-3). Two broods collected at Smooth Pools in 2005 (smp32 and plan03) came from parents that were homozygous for allele 232 at locus A110, and included both homozygous offspring (232/232) plus one (smp32-3) or two (plan0304, plan0307) heterozygotes (228/232). All other broods consisted of parents and offspring that were homozygous at both A110 and C212. The segregation of multiple alleles among brood-mates in all three of these broods is consistent with conventional sexual reproduction in *P. parvivipara* rather than some form of amictic parthenogenesis. The inheritance of non-maternal alleles in broods smp36 and plan03 (in both cases, alleles observed in other adults in population samples) strongly implies outcrossing rather than self-fertilization of at least some internally fertilized offspring.

**Brood Allometry**

There was a positive and highly significant relationship between parent size and clutch size in *P. parvivipara* ($R^2 = 0.35$, $P = 3.425e^{-9}$). This data suggests that, as expected, larger parents have larger clutches (as in Byrne 1996).

The presence of sibling brood cannibalism in *Parvulastra* implies a relationship between clutch size and offspring size such that as clutches become smaller (possibly due to cannibalism) the offspring remaining in the brood (possibly cannibals) become increasingly large from nutrients gained from siblings. If larger parents with higher fecundity cannot also provide greater resources for their more numerous offspring (in the form of security from sibling

cannibals), then larger parents may suffer a proportionately greater loss of offspring, and such a relationship would help to explain the evolution of brooding only among the smallest asterinid species. I found a negative relationship between clutch size and average offspring size, such that as offspring become larger clutch size decreases. This relationship was not significant when compared among all broods ($R^2$ = 0.012, P = 0.3), nor was the relationship between variance in average offspring size vs. clutch size ($R^2$ = 0.002, P = 0.29). However, when parent size was included as a covariate in the linear model, the relationship between clutch size and average offspring size was highly significant as was the interaction between offspring size and parent size (overall: $R^2$ = 0.40, P = $1.894e^{-9}$, interaction: P = 0.024), suggesting that the ability of average offspring size to explain clutch size depended on the sizes of the parent. In this case, as parent size increases the relationship between offspring size and clutch size becomes stronger and more significant.

I also examined the relationship (Figure 5.2) by binning the data into four parent size classes (see methods for categories). Overall there was a significant difference among the slopes of the four size classes (*F* = 2.7, P = 0.04). In the two small parent size classes (3.0-4.8mm and 5.0-5.9 mm) the relationship between offspring size and clutch size was negative but not significantly different from zero ($R^2$ = 0.08 P = 0.1 and $R^2$ = 0.06 P = 0.2 respectively). In contrast, the relationship between average offspring size and clutch size had a steep negative slope and was highly significant ($R^2$ = 0.39 P = 0.001) in the second to largest parent size class and a non-significant but large negative slope ($R^2$ = 0.26, P =

0.089) among the largest size class (probably due to small sample size, n = 12). These results suggest that larger parents suffer greater loss from sibling cannibalism than the smaller parents.

In an ANOVA analysis to determine whether population of origin explained a significant proportion of the variation in average offspring size, clutch size or parent size, I found that population only explained a significant proportion of the variation in parent size ($P = 8.01e^{-5}$). Upon closer examination I found that parents from the Smooth Pools population sampled in 2007 were significantly larger then either Smooth Pools 2005 or The Granites. Since population explained a significant proportion of the variation in parent size I ran another linear model of the relationship between clutch size and average offspring size and included both parent size and population of origin as covariates. I found that population of origin did not show a significant interaction with average offspring size to explain the variation in clutch size (P = 0.32 for SMP2007 and 0.58 for TGR), and that parent size still showed a marginally significant interaction with average offspring size in explaining the variation in clutch size (P = 0.07). Furthermore, when I examined the relationship between clutch size and average offspring with only population as a covariate, I found that the overall relationship between average offspring size and clutch size becomes non-significant ($R^2$ = 0.07 P = 0.2) and that there is no significant interaction between population of origin and offspring size (P = 0.92 for SMP2007 and 0.06 for TGR).

# .Discussion

### Mating Systems in *P. vivipara* and *P. parvivipara*

Despite the strong inbreeding in *P. vivipara* and *P. parvivipara* populations (low allelic diversity, few heterozygotes, skewed allele frequency distributions) and limited scope for finding heterozygous parents or non-maternal alleles among their offspring, I found some evidence for outcrossing in *P. parvivipara.* I detected non-maternal alleles in a total of 3 individuals (out of 181, or about 1.6%) from two broods (out of 42, or about 4.7%). Both out-crossed broods were from the Smooth Pools population, which had the highest level of allelic and genotypic variation of all populations of *P. parvivipara* (or *P. vivipara*) studied in Chapter 4, suggesting that I was able to find evidence of outcrossing only in broods from the population where it was most likely to be detectable, and that the frequency of out-crossed offspring might be much higher than the 1-5% range suggested above. Low allelic and genotypic diversity in most populations prevents me from comparing estimated outcrossing rates among them. Similarly, the identification of the segregation of alleles from a heterozygous parent into both of the homozygous offspring genotypes, along with evidence of outcrossing in some broods, indicates that this species is undergoing sexual reproduction. However, I cannot completely rule out asexual production of some offspring via cloning or parthenogenesis due to the high frequency of homozygous genotypes among female parents, their offspring, and the populations as a whole (see Sunday et al. 2009)

**Analysis of Brood Allometry and Sibling Conflict in *P. parvivipara***

The strong negative relationship between average offspring size and clutch size for large parents but not for small parents suggests that large parents lose more from brooding and sibling cannibalism then smaller parents. Specifically, because there is cannibalism within the broods, and all broods (regardless of parent size) converge toward a few large offspring, larger parents that have larger clutches in the beginning lose more offspring to cannibalism than small parents that have smaller clutches.

Evidence from my microsatellite population genetics and the brood analysis suggests that most pairs of broodmates are genetically identical to one another. It is therefore unlikely that offspring are selectively cannibalising half siblings rather than full siblings because they should have little means to measure their relatedness to clutch-mates (Pfennig 1997), and it is more likely that positioning of an individual within the brood chamber, and its age and relative size, dictates the success or failure of particular individuals within the clutch (Polis 1981, Polis 1992).

Although evidence from microsatellite data suggests that individuals are highly inbred with little genetic variation, other classes of molecular markers that may be more highly variable, such as nuclear sequence data from highly variable regions of the genome, may reveal higher levels of outcrossing or segregation of alleles in heterozygous parents in the production of clutches. Furthermore, understanding the true nature of sibling recognition within clutches would benefit

from the identification of more outcrossing events among clutches and warrants further sampling of broods throughout the range of *P. parvivipara.*

**Mating Systems Evolution in *Parvulastra***

Evidence of outcrossing in *P. parvivipara* implies a mixed mating system with a potentially high degree of self-fertilization and at least some outcrossing. However the ecological advantages to selfing vs. outcrossing within this species are unknown, therefore I can only speculate on the fitness effects and evolutionary trajectory of the mating system of *P. parvivipara*.

Mixed mating systems, where individuals are capable of self-fertilization and out-crossing, are sometimes thought of as an evolutionary pathway to pure self-fertilization (Jarne and Charlesworth 1993). However these mixed mating systems can be evolutionarily stable in many different ecological and evolutionary conditions, in which the combination of selfing with outcrossing (e.g. some degree of recombination among gametes) is optimal (Goodwillie et. al. 2005). Several possible models for the maintenance of a mixed mating system have been put forward, including the influence of bi-parental inbreeding (Uyenoyama 1986), pollen (sperm) discounting (Holsinger 1991) and the interaction of dispersal distance and inbreeding depression (Holsinger 1986). Although all of these models provide viable explanations for the maintenance of mixed mating, few have been empirically tested (Goodwillie et al. 2005). Furthermore, in situations where extreme philopatry of self-fertilized and outcrossed offspring occurs, conflicts among siblings or the presence of kin recognition can further

influence the evolution of mating systems (Waller 1980, Clay and Antonovics 1985, Keough 1984, Grosberg and Quinn 1986, Elgar and Crespi 1992).

Studies of selfing and outcrossing rates using *F* statistics and analyses of mating systems using progeny arrays have been conducted in a variety of animal and plant species. Analyses of mating systems in invertebrates include, crustaceans (Toonen 2004, Gosselin 2005), molluscs (Shaw and Saur 2004) insects (e.g. Good et al. 2006), cnidarians (Edmands 1995, Sherman 2008), ascidians (Cohen 1996, Johnson and Yund 2007), and echinoderms (Boissin 2008) and these studies show that high levels of multiple paternity (e.g. Johnson and Yund 2007), a high prevalence of self-fertilization (e.g. Edmands 1997), or mixed mating systems can exist in nature. However, the literature on mating systems in invertebrate animals lags behind that of terrestrial plants (Jarne and Auld 2007) and a more thorough understanding of the genetic relationships among progeny, and of brood characteristics in invertebrate animals will bring me closer to understanding the evolution and maintenance of mating systems in sexual organisms (Carlon 1999).

My analysis of *P. parvivipara* builds on upon previous research by documenting the presence of both self-fertilization and outcrossing in a brooding echinoderm. One argument in support of the fact that this species is transitioning from a gonochoric mating system to self-fertilization is that its congener *P. exigua* is a predominantly outcrossing protandric species capable of a small amount of self-fertilization (See chapter 1, Byrne 1995, 1996). The life history of *P. parvivipara,* which includes a potentially high rate of self-fertilization may have

had a fitness advantage in the ancestral live bearing *Parvulastra* because of the ecological conditions that populations encountered: life in the high intertidal with no ability to spawn in to the water column, and isolation of few individuals on the periphery that, in order to reproduce, had to self fertilize.

**Limitations and Caveats**

One limitation on my analysis is the overall low variability in the microsatellite loci. The low levels of polymorphism in all loci that amplified in live-bearing asterinids (and that were polymorphic in non-live bearing species) suggest that the levels of variability in these loci may reflect the fact that these species are highly inbred. In *P. vivipara* only one of the three loci genotyped was polymorphic and consisted of only two alleles at that locus with a highly skewed frequency distribution. Both loci sampled for *P. parvivipara* were polymorphic, however diversity at each of these loci also was limited to one common and one rare allele. The downfall of genotyping loci with such low variability is that I cannot infer the true outcrossing rate within broods (Jarne and David 2008). This is especially true if there is a significant amount of bi-parental inbreeding occurring in these populations: outcrossing between individuals of similar genotype cannot be distinguished from self-fertilization. In this case, adults of both *Parvulastra* species could have a much higher outcrossing rate than suggested by this study and the lack of polymorphism prevents the detection of these outcrossing events.

Another caveat is that the broods that could not be completely genotyped for this analysis could have null alleles segregating in the population, which

would prevent the detection of polymorphisms segregating in the population. These unobservable polymorphisms could be segregating in out-crossed individuals that are not being identified by this study. Because I did not observe null-null homozygotes among population samples of adults, the frequency of such null alleles is probably low and it is therefore unlikely that many of the broods I sampled were the offspring of outcrossing with males bearing null alleles.

**General Conclusions**

My research on mating system variation in the Asterinidae is the first to empirically identify outcrossing among the live-bearing hermaphroditic asterinids. There has been some speculation on the potential for outcrossing (Dartnall 1965, Byrne 1996a, Prestedge 1998), but this research is the first to document the phenomenon, admittedly only in two clutches. Although *P. parvivipara* occur in dense aggregations in the field, the mechanistic obstacles to successful outcrossing still seem significant. Conspecific sperm must swim through the gonophore of the 'male' adult and enter the 'female' via the gonophore, some other opening (the maderoporite or the mouth), or perhaps through the body wall of an adjacent adult to find a single unfertilized ovum. It is therefore highly surprising that any outcrossing occurs in this species.

Finally, this study provides one possible solution to the question of why small species brood (Strathmann and Strathmann 1982). Larger adults that produce more gametes and offspring ultimately give birth to similar numbers of larger-sized juvenile offspring compared to smaller parents with smaller early

clutch sizes, and thus have a greater net decrease in clutch size relative to smaller parents. Interestingly, Strathmann and Strathmann (1982) emphasized the potential for differential predation in larger vs. smaller clutches of offspring. In this case, larger clutches (produced by larger parents) have higher sibling-mediated predation, thus conferring lower fitness relative to the fecundity of larger brooding adults.

**Figure 5.1 *Parvulastra parvivipara* brood juveniles and parents. Adults with aboral surface removed. Offspring are brooded in the interambulacral spaces.**

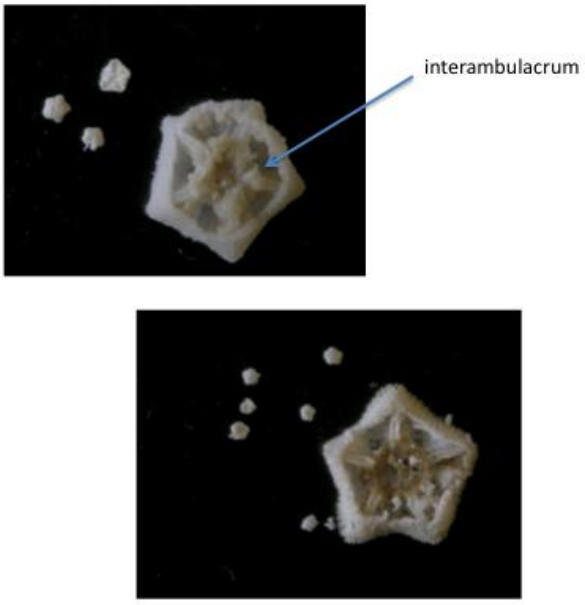
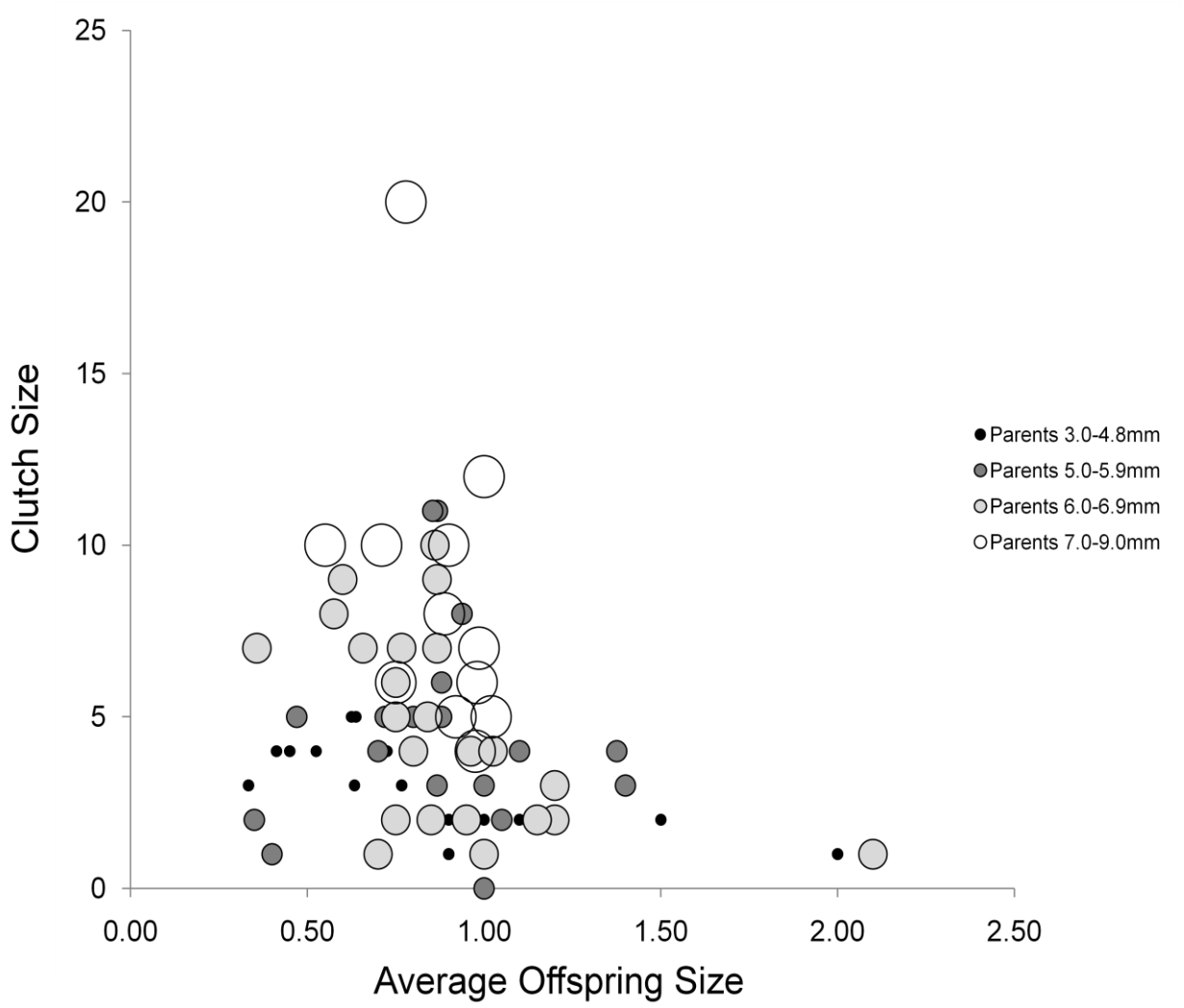


**Figure 5.2 Clutch size plotted against average offspring size. Adult (parent) size classes are indicated by the different sized circles. The smallest circles indicate the smallest parents and the largest circles indicate the largest parents.**



# Chapter 6 **SOMETHING FOR NOTHING? RECONSTRUCTION OF ANCESTRAL CHARACTER STATES IN ASTERINID SEA STAR DEVELOPMENT**

This chapter was modified from Keever and Hart 2008. Something for Nothing? Reconstruction of Ancestral Character States in Asterinid Sea Star Development. Evolution and Development 10: 62-73, with permission from Wiley-Blackwell Publishing.

## .General introduction

Comparative analyses using phylgoenetically independent contrasts rely on accurate reconstructions of ancestral character states in order to examine the correlated evolution of phenotypic characters. Without the appropriate model of character evolution, ancestral states along nodes of a phylogeny can be incorrectly reconstructed biasing the results of independent contrasts among characters. In this chapter I utilize Bayesian based methods of ancestral character state reconstruction to test multiple models of the evolution of asterinid dispersal mode and mating systems along a phylogenetic tree. I examine the influence of the model of character evolution on ancestral character state reconstruction and finally I speculate on the utility of ancestral character state reconstruction in cases where no evidence based on first principles exists for the model of character evolution.

## .Abstract

Traits from early development mapped onto phylogenetic trees can potentially offer insight into the evolutionary history of development by inferring the states of those characters among ancestors at nodes in the phylogeny. A key and often-overlooked aspect of such mapping is the underlying model of character evolution. Without a well-supported and realistic model ('nothing'), character mapping of ancestral traits onto phylogenetic trees might often return results ('something') that lack a sound basis. Here I reconsider a challenging case study in this area of evolutionary developmental biology: the inference of ancestral states for ecological and morphological characters in the reproduction and larval development of asterinid sea stars. I apply improved analytical methods to an expanded set of asterinid phylogenetic data and developmental character states. This analysis shows that the new methods might generally offer some independent insight into choice of a model of character evolution, but that in the specific case of asterinid sea stars the quantitative features of the model (especially the relative probabilities of different directions of change) have an important effect on the results. I suggest caution in applying ancestral state reconstructions in the absence of an independently corroborated model of character evolution, and highlight the need for such modelling in evolutionary developmental biology.

## .Introduction

Comparative phylogenetic analysis of ecological, morphological, and genetic features of early development seems to hold considerable promise for revealing pathways of phenotypic evolution (Knoll and Carroll 1999), such as parallelisms (Richmond 2006), atavisms (Collin and Cipriani 2003), or correlations among traits (Pagel and Meade 2006). Mapping of developmental traits onto the tips of well-resolved phylogenetic trees seems intuitively likely to lead to important insights (Serb and Oakley 2005). A familiar example is segmentation in embryos of annelids and arthropods. Conclusive evidence in support of surprising phylogenetic relationships (Giribet 2003) among annelids, arthropods, and non-segmented bilaterians – combined with descriptive or manipulative studies of the genetic and embryological basis of the shared similarity (Seaver 2003; Seaver et al. 2001) – led to a novel hypothesis of deep homology of the underlying gene networks (but not perhaps of the morphogenetic processes) responsible for segmentation (Erwin and Davidson 2002).

Comparative analyses of development implicitly assume – or search explicitly for – the character states of ancestors represented by nodes in the tree of life. Inferring ancestral character states minimally requires sound knowledge of the states of these developmental characters among extant taxa and the phylogenetic relationships among those taxa. In addition, the quality of ancestral state inference depends crucially on the underlying model of character change, including the direction, order, and reversibility of state changes (Strathmann 1993; Mooers and Schluter 1999; Hart 2000; Collin and Cipriani 2003; Pagel

2004; Nosil and Mooers 2005). These models can be thought of as quantitative estimates of the probability that apparently similar character states shared among two or more taxa are homologous or analogous. These models are usually based on information other than phylogenetic relatedness, but can later be used to map character changes onto phylogenetic hypotheses of the relationships between the extant organisms and their character states. The simplest of these models are unconstrained parsimony or Brownian motion models that assume equally likely changes in character states in any direction. A sound model of character evolution is important for ancestral character state reconstruction because (in combination with the phylogeny and the tip distribution of character states) it is essentially sufficient to specify the evolutionary history of character states from root to tips of a clade (Pagel et al. 2004).

This issue of the model of character evolution in ancestral state reconstruction has a long parallel history in the development of cladistic and phylogenetic methods of inferring evolutionary trees (Farris 1977; Felsenstein 1978; Yang 1996). The view of unconstrained parsimony phylogenetic methods as 'model-free' (and therefore preferable for making few assumptions) gradually gave way to the realization that such methods merely employed relatively simple models with little biological realism in comparison to the parameter-rich maximum likelihood models that are now routinely applied to DNA or protein sequence data (Thorne et al. 1992; Felsenstein 2001). The related field of reconstructing ancestral protein sequences and then synthesizing those ancestors in vitro (e.g., for studies of the evolution of enzyme structure and function) has adopted the

same methods, and thus employs a realistic biological basis for inferring ancestral character states (Williams et al. 2006).

Unlike the reconstruction of ancestral protein sequences, most reconstructions of ancestral states for developmental (or other phenotypic) characters lack an underlying model derived from experimental or descriptive observations independent of the comparative analysis. These models are hard to derive because the characters are often complex and the mechanisms by which new character states arise are usually not well known. In the absence of well-corroborated independent models of character evolution (e.g., from the fossil record or from shared ancestral polymorphisms; Finarelli and Flynn 2006; Igic et al. 2006) the application of these ancestral character state reconstruction methods based on unconstrained parsimony or Brownian motion models might still be used to estimate ancestral character states with more or less statistical confidence. However, this approach has been criticized as ‘something for nothing’ (e.g., Strathmann and Eernisse 1994) because alternative biologically plausible models of character evolution may lead to different inferences of ancestral character states and the direction and frequency of state changes over time. Without the prior independent derivation of an appropriate model (‘nothing’), the ancestral character states (‘something’) inferred under a simplistic parsimony or Brownian motion model might be deeply flawed.

In spite of the well-known importance of the underlying model of character state change, methods for ancestral character state inference are often applied to cases in which it is not possible to specify a prior model (e.g., Brumfield and

Edwards 2007; Leschen and Buckley 2007). In these cases, both the model of character evolution (the relative probabilities of particular state changes) and the probabilities of specific ancestral states are inferred together from the same set of data (phylogeny and character states of extant taxa). This approach has become a popular tool for peering backward in time to examine the history of developmental, phenotypic, and biogeographic changes that are not observable, for example, in the fossil record (e.g., Richmond 2006).

Here I reconsider a difficult problem in ancestral state reconstruction: larval forms of asterinid sea stars. I use the best available methods (Pagel et al. 2004; Pagel and Meade 2006) to explore the consequences of different model constraints on character evolution for inferring both the best-fit model parameters and the ancestral states of larval characters in asterinid development. I focus on three specific characters singly (as a series of two-state characters) and together (as one composite four-state character). I contrast the inferred best models and ancestral character states in these four analyses against a simple heuristic model based on apparent morphological and ecological synapomorphies and symplesiomorphies in reproduction and early development among asterinids and between asterinids and other sea stars or other deuterostomes. I show that the new methods offer some objective statistical basis for choosing among models, but the choice of models is still crucially important because different models lead to different ancestral state reconstructions. This choice is still mostly unconstrained by any independent quantitative model of the rate, order, and direction of changes in these early developmental characters.

## .Background

Asterinid sea stars include a wide variety of larval forms and developmental patterns (Chia and Walker 1991; Byrne 2006). Morphological and ecological characteristics of early development covary among species as four different suites of character states or modes of development (Strathmann 1985) (See description in Chapter 1). For the purposes of this chapter life history modes are coded as follows 0 = planktotrophy, 1 = lecithotrophy, 2 = benthic egg mass depositing, and 3 = live bearing.

Phylogenetic relationships among asterinid lineages imply a large number of evolutionary changes in modes of development among relatively small numbers of branch points in the phylogeny (Hart et al. 1997; Waters et al. 2004). One approach to inferring the pattern and timing of evolutionary changes in asterinid development has compared the phylogenetic distribution of modes of development against a simple heuristic model based on assumptions about homologies among these modes of development across lineages (Jagersten 1972; Strathmann 1978; Nielsen 1998; Wray and Raff 1991; Wray 1996; Strathmann and Eernisse 1994; Strathmann 1985; Smith 1997; Hart et al. 1997; Hart 2000, 2002; McEdward and Miner 2001; Jeffrey and Emlet 2003). This heuristic model has been conceived as a series of ordered and irreversible changes from planktotrophy (0) as the plesiomorphic ancestral condition to lecithotrophy (1) by loss of adaptations for larval feeding; from planktonic lecithotrophy (1) to benthic development (2) by the evolution of encapsulation or external brooding; and from external (2) to internal (3) benthic development by

the evolution of internal fertilization and live birth. The loss of complex structures associated with the transition to obligate lecithotrophy (0→1) and to internal development (2→3) has been used to argue against the reversibility of some of these transitions (Strathmann 1978; Byrne 1996; Byrne and Cerra 1996). This heuristic model particularly emphasizes the strong apparent homology among planktotrophic larval forms. The complexity and conserved nature of the ciliary feeding mechanism and the digestive structures of bipinnaria (or other dipleurula-like) larvae between classes and phyla suggests that this larva was present in a common ancestor of asterinids and other sea stars, and that several independent gains of this larval type are much less likely than the parallel evolution of lecithotrophy, benthic development, or viviparous brooding among different lineages.

An alternative approach is based on fitting ancestral character states to nodes (and different rates and directions of state changes along branches) of a phylogeny under some minimization function to find the combination of states, directions of change, and rates of change that require the smallest number of state changes. Several studies of asterinids have used a series of progressively more sophisticated minimization functions, from simple parsimony to more complex maximum likelihood methods (Hart et al. 1997; Cunningham 1999; Huelsenbeck et al. 2003). However, a large number of differences in modes of development among a small number of asterinid lineages has typically led to weak statistical support for many inferred ancestral states and numerous conflicts between states inferred from optimization methods versus states that would be

inferred under the ordered irreversible heuristic model. For example, the heuristic model assumes that planktotrophic larval forms (0) and their underlying morphogenetic processes are shared ancestral similarities among the asterinid lineages with this mode of development. However, optimization methods consistently infer planktonic lecithotrophy (1) or benthic embryos (2) as the ancestral mode of development, with one or more reversals to planktotrophy in asterinid lineages that have been deeply nested within clades of close relatives with lecithotrophic or benthic development.

Evolutionary problems of this nature exist in many closely related taxa for many different types of characters (Wray 1996; Donoghue et al. 1998; Omland 1999; Hart 2000; Igic et al. 2006), and have provided the motivation for analytical methods that estimate ancestral character states and predict a model of character change on a phylogeny (Schluter et al. 1997; Cunningham et al. 1998; Pagel 1999; Mooers and Schluter 1999; Schultz and Churchill 1999; Lewis 2001; Huelsenbeck et al. 2002; Huelsenbeck et al. 2003). These methods have proved especially useful when investigating patterns of evolution of a single character, or examining the correlated evolution of two characters during the phylogenetic history of a clade (Cunningham 1999; Collin 2001; Jeffery and Emlet 2003; Duda and Palumbi 2003; Nosil and Mooers 2005).

Careful reconsideration of the heuristic model of asteroid life history evolution (McEdward and Janies 1993, 1997; McEdward 2000; McEdward and Miner 2001) has not yet led to an independently derived model of character state change. In the absence of such a model of developmental evolution, is the

reconstruction of asterinid ancestral developmental states an intransigent problem? Or could recent improvements in the available data and analytical methods allow me to infer both the history of evolutionary changes in asterinid modes of development and the most probable model of character state change at the same time? The original mtDNA phylogeny (Hart et al. 1997) has been significantly expanded by Waters et al. (2004) (see also Hart et al. 2003), and many of the lineages added to the phylogeny have well-characterized modes of development (Byrne 2006). These include three planktotrophic species from divergent clades that each include other species with lecithotrophic or benthic development. The inclusion of these additional planktotrophic lineages could lead to a higher probability of inferring planktotrophy as the ancestral mode of development among asterinids. New Bayesian methods of ancestral state reconstruction (Pagel et al. 2004; Pagel and Meade 2006) include several significant improvements (see below).

Our primary motivation for reconsidering these questions is to test the recent and widely applied Bayesian methods against a well-known problem in ancestral state reconstruction. A secondary motivation more specific to the analysis of development and evolution is to reconstruct life history character states in asterinid sea stars as part of a broader comparative study of population-level characters such as inbreeding coefficients, population genetic differentiation, and genetic effective population size that are predicted to covary with life history characters (e.g., Bohonak 1999). Because an independently corroborated model of life history evolution is not known for asterinids, an

obvious first step in this comparative study is to determine how robust the inferred ancestral states might be to differences in model assumptions. Can this combination of new data and new methods produce improved inferences of ancestral states for asterinid developmental characters? And do these improved inferences still conflict with simple ideas about homologies among modes of development, especially with the notion that complex planktotrophic larval forms are lost irreversibly or shared as symplesiomorphies but never gained by reversal?

## .Methods

I used mitochondrial tRNA and COI data from 28 species of asterinid sea stars with known modes of development to construct a Bayesian posterior distribution of phylogenetic trees (Hart et al. 1997; Waters et al 2004; Byrne 2006). Asterina species were used as the outgroup to other Asterinidae (Hart et al. 1997). I used Modeltest to find the best-fit model of sequence evolution (Posada and Crandall 1998). I then sampled the posterior distribution of trees under that model in BayesPhylogenies (Pagel et al. 2004). I ran multiple chains during multiple runs to ensure convergence on a single posterior distribution of trees. Furthermore I ensured that the program was effectively exploring the likelihood surface of phylogenies by evaluating the autocorrelation coefficient between different trees sampled throughout the analyses. I discarded the first 25% of the total MCMC to avoid sampling trees while the search process was

climbing the likelihood surface. I found strong support for all nodes in the resulting tree (>90% posterior probabilities for all clades).

I analyzed three two-state characters (associated with the four asterinid modes of development): larval nutrition as planktotrophy (coded as 0) or some maternal source of nutrition (coded as 1); larval habitat as planktonic (0) or benthic (1); and site of development as external (0) or internal (1) to the parent. I also analyzed a single four-state character in which the states represented the four modes of development summarized above (0-3).

I reconstructed ancestral states for each character in Bayestraits (Pagel et al. 2004; Pagel and Meade 2006). This program uses a continuous-time Markov model of character state changes over very small intervals of time that are independent of the previous interval or state of the character (Pagel 1994).

I mapped each character onto our posterior distribution of 1000 topologies and branch lengths. For each iteration of each Bayesian reconstruction, Bayestraits draws a random set of values (within the limits of prior specified values and the constraints of the model of character evolution) for the probability of change from one character state to another, and creates a reconstruction of ancestral states based on these values on the tree topology that was chosen from the search. The algorithm accepts the reconstruction on this set of rates and on this topology based on a Metropolis Hastings importance sampling. This search algorithm always accepts more probable reconstructions (that better fit the tip distribution of character states to the topology and branch lengths) and accepts less probable reconstructions in proportion to their probability (Metropolis

et al. 1953; Hastings 1970). This sampling process occurred in an interval of one sample every 300 iterations of the run, and a resulting posterior distribution of models and their associated likelihood values were stored in the output. I ran each search twice for 300 million iterations to ensure that the process converged on a stable range of likelihood values with similar probabilities of ancestral states and rates of character change between the duplicate searches.

I used both gamma-distributed hyperprior and uniform prior distributions of the transition probabilities between states. I report results from the hyperprior gamma distribution because the results were similar to those using the uniform prior distribution and because the hyperprior provides more flexibility when implementing the prior distribution values. The hyperprior is seeded from a uniform distribution and evaluated in a gamma distribution (Pagel and Meade 2006). A gamma distribution was used for the assigned priors because the probabilities of change in life history characters are moderate to high in this group (Huelsenbeck et al. 2003), and the right-skewed gamma tail allows for slightly larger values to yield higher probabilities than in a normal distribution.

I compared four models of evolution for each two-state character. The first was a conservative null model (N) with a single transition probability (q) for any character state change ($q0\rightarrow1 = q1\rightarrow0$) that I tested against three alternative hypotheses (Mooers and Schluter 1999; Cunningham 1999). For convenience, and to distinguish between transition probabilities and the probabilities of specific ancestral states at nodes, I refer to the estimated qij values as rates, though these are formally probabilities of state change. The first alternative was a free

model (Af) that allowed both transition probabilities to vary as a posterior distribution of rates. Under this alternative model, the transition probabilities were estimated as the most probable Bayesian fit to the tree topology, branch lengths, and tip distribution of character states. The second alternative was a heuristic model (Ah) that excluded one class of state changes (q1→0 = 0) and allowed the other transition probability (q0→1) to be freely estimated as a posterior distribution of transition rates (similar to Af). This model incorporated the assumptions of the heuristic model of the evolution of modes of development based on assumed morphological and ecological symplesiomorphies among larval forms. The third alternative emphasized so-called character state reversals (Ar): this model was similar to the heuristic alternative model (Ah) but excluded the other class of state changes (q0→1 = 0), and freely estimated the transition probability (q1→0) that is assumed to be zero under the heuristic model.

For the four-state character, I tested a null model (N) with all 12 transition probabilities (qi→j) equal against three alternatives hypotheses. The first alternative (Af) was similar to the free alternative (Af) for the two state-characters in that each of the 12 possible transition probabilities was allowed to vary freely of the others and was independently estimated as a posterior distribution in the Bayesian run. The second alternative hypothesis was an ordered and reversible model (Ar) of six transition probabilities among character states (q0→1,q1→0, q1→2, q2→1, q2→3, q3→2) estimated as posterior distributions. The third alternative was an ordered and irreversible alternative model (Ah) with three transition probabilities freely estimated (q0→1, q1→2, q2→3) and all others set

to zero. This alternative incorporates assumptions about the ancestral mode of development (planktotrophy), and the irreversible loss of feeding and planktonic larval forms, that are key features of the heuristic model of evolution of modes of development. Comparisons among these models for the four-state character allowed me to explore, for example, the importance of reversibility in the transition between planktonic and benthic development that has been proposed for echinoderms in general (McEdward and Janies 1997) and some sea stars in particular (McEdward 1995; Hart et al. 2003).

A Bayes factor test (Pagel and Meade 2006) was used to evaluate the difference between the posterior distribution results of the null and alternative models of evolution for each character. This test is similar to other hypothesis tests such as the Bayesian information criterion and the likelihood ratio test. The Bayes factor test is based on the marginal likelihood (margL) of the null and alternative models. These marginal likelihood values are found by taking the harmonic mean likelihood value from the posterior distribution in the Bayestraits analysis. I used the harmonic mean of the likelihood values rather than the arithmetic mean because it more closely approximates the mean of the posterior distribution of likelihood values obtained in the reconstruction. I calculated the Bayes factor as 2 x (ln margL(alternative) - ln margL(null)). In general, a Bayes factor value > 2.5 is considered sufficient to reject the null model (see Pagel and Meade 2006). The “best model” of character evolution is the model that, when tested against the null model (N), gives the largest increase in the marginal likelihood value that is > 2 Bayes factors. If no model has a substantially higher

marginal likelihood than the null model (N) then the null model and its parameter estimates are considered to be the best fit.

I illustrate the results of our model comparisons as simple path diagrams (Fig. 1). I also show reconstructions for character states at all nodes drawn on the 50% majority rule consensus phylogeny for the most probable model and for the heuristic alternative model (Ah) for each of the four analyses (Figs. 2-5).

## .Results

### Larval nutrition: gain and loss of planktotrophy

The best model of character evolution was model N, the null hypothesis of equally likely gains and losses of a feeding larval form ($q0 \rightarrow 1 = q1 \rightarrow 0 = 6.3$; Figure 6.1A). The marginal likelihood for the free model (Af) was slightly lower than the null model (with a small negative Bayes factor). Only the non-heuristic alternative model (Ar), which emphasizes apparent character reversals, was associated with a better marginal likelihood, but the improvement of this model fit was modest (Bayes factor = 1.76) and not large enough to consider rejecting the null model. The heuristic alternative model (Ah) was associated with a substantial decrease in marginal likelihood and a higher rate of character changes ($q0 \rightarrow 1 = 11.7$).

In general, ancestral states reconstructed under the null model (N) were similar to the ancestral states under the heuristic alternative (Ah) model at shallow nodes in the phylogeny, but different (and often with high associated

probabilities) at many deeper nodes (Figure 6.2). This result implies strongly conflicting inferences of ancestral states under these two models. In particular, this result implies uncertainty about the origin and history of feeding bipinnaria larval forms in *Asterina, Patiria, Meridiastra, and Patiriella*. One model (N) represents our best insight into the mode of evolution for this character as inferred from the phylogenetic distribution of character states under a Bayesian simulation (where these bipinnaria forms may be convergently evolved from a strongly inferred ancestral nonfeeding larval form), while the other model (Ah) represents our best intuition about homologies among bipinnaria forms (where these four genera share the bipinnaria as a symplesiomorphy for sea stars). For example, the most recent common ancestor of *Patiria and Patiriella* species is strongly inferred (86%) to have non-feeding larval development (1) under the null model (N), but under the heuristic model (Ah) the ancestor at this node is strongly inferred (99%) to have been planktotrophic (0).

**Larval habitat: gain and loss of planktonic dispersal**

The best model of character evolution was the free alternative (Af), in which the rate of evolution to benthic development ($q_{0\rightarrow1}$ = 41.8) was high but only about half the rate of evolution to planktonic dispersal of embryos and larvae ($q_{1\rightarrow0}$ = 94.1; Figure 6.1B). This result is consistent with the relative frequency of planktonic and benthic development among the terminal taxa. Model Af was associated with a higher marginal likelihood than the null model (N) by a relatively large Bayes factor (2.7). Like our analysis of planktotrophy, the heuristic alternative model (Ah) was a substantially poorer fit to the data, with a lower

marginal likelihood, a large negative Bayes factor, and a low rate of character state change ($q_{0\rightarrow1}$ = 3.7) in comparison to the free alternative model (Af).

In contrast to our analysis of planktotrophy, larval habitat was reconstructed with low probabilities – in the range of 50% – for the most probable character state under the best (Af) model (Figure 6.3). Larval habitat was reconstructed with much higher probabilities – in the range of 90% – under the heuristic alternative (Ah). In general, across all four of our character reconstructions (particularly reconstructions of larval habitat, site of larval development, and the four-state 'modes of development' character, see below), models with fewer restrictions on character evolution (such as Af) returned higher rates of character state change and lower probabilities of ancestral states (e.g., Vanderpoorten and Goffinet 2006).

**Site of development: evolution of viviparity**

The most probable model of character evolution was the free alternative model (Af) with high rates of evolution (q0→1 = 65.2) and loss (q1→0 = 307.5) of live bearing (Figure 6.1C). Model Af was more likely than the null model (N) by a very large positive Bayes factor (12.5). Unlike the other analyses of two-state characters, the heuristic alternative model of character evolution (Ah) was more probable than the null model of character evolution (N) (Bayes factor = 2.0) but lost the Bayes factor tug-of-war to a considerably better alternative model (Af).

Like the analyses above, ancestral character states were reconstructed with low probabilities under the best model (Af) and high probabilities under the

less likely heuristic alternative (Ah) (Figure 6.4). This pattern was consistent both in clades that included a single character state among the tip taxa (e.g., *Patiria* + *Meridiastra*) and in clades with both external (0) and internal (1) development (e.g., *Cryptasterina, Parvulastra*). This similarity across clades suggests that the low probabilities associated with the most probable ancestral state are largely driven by the globally high inferred rates of character state change (see above) under the most probable model (Af) compared to the heuristic model (Ah; $q0 \rightarrow 1 = 2.1$), and not by the local tip distribution of character states among subsets of extant taxa.

**Four modes of development**

The best model of evolution for this four-state character was the one-rate null model (N) with $qi \rightarrow j = qj \rightarrow i = 18.3$ (Figure 6.1D). I could not reject this null when tested against the 12-rate free alternative model (Af), for which the marginal likelihood was slightly better (-35.7) but with a modest Bayes factor (0.4), nor when I tested model N against either the ordered and reversible alternative model (Ar) or the ordered and irreversible model (Ah). This last alternative model reflects the intuition that changes in these modes of development might not be easily reversed, but this model fit to the posterior distribution of trees had a substantially lower marginal likelihood value than the null model (Bayes factor = -2.8).

Like our reconstructions of larval nutrition (planktotrophy) above, our reconstructions of the four-state character under the null (N) and heuristic

alternative (Ah) models were similar at most shallow nodes but different (and with high associated probabilities) at some deeper nodes (Fig. 6.5). The most striking examples of differences were the recent common ancestors for members of two clades (*Asterina*, *Parvulastra*) that include benthic development. These ancestral states were reconstructed with high probability as planktotrophic (*Asterina*; 100%) or benthic egg masses (*Parvulastra*; 91%) under model Ah, and with lower probability as benthic egg masses (*Asterina*; 61%) or viviparity (*Parvulastra*; 57%) under model N.

At three deeper nodes (the most recent common ancestors among *Patiria*, *Meridiastra, Patiriella*, and *Aquilonastra* species), model N indicated planktonic lecithotrophy (1) as the ancestral character state with probabilities of 67-79%, but model Ah indicated planktotrophy (0) with higher probabilities of 99% in all three cases. Differences in ancestral states at some shallower nodes seemed to reflect differences at deeper nodes. For example, model N weakly (57%) supported viviparity (3) for the most recent common ancestor of *Parvulastra* species, which was in turn descended from an ancestor that may (55%) have had planktonic lecithotrophic development (1) at the next deeper node. In contrast, model Ah strongly (91%) supported development in benthic egg masses (2) as the ancestral state for the most recent common ancestor of *Parvulastra* species, and planktotrophy (0) for the ancestor at the next deeper node.

One shallow node is particularly worth noting in this context. Both models N (81%) and Ah (68%) supported planktonic lecithotrophy (1) as the most probable state for the most recent common ancestor of *Meridiastra* species,

including *M. mortenseni* with planktotrophic development (0). In this case, the two models were in surprising agreement that planktotrophy in *M. mortenseni* might be a recently evolved character state. Under the heuristic model, such a reversal should not be found, but this particular result may not be conclusive. First, the posterior probability under model Ah was low (68%) relative to the probabilities usually considered reliable in other Bayesian reconstructions. Second, the cause of this specific anomaly appears to be a combination of some topological uncertainty about the relationships of *M. mortenseni* plus a strong bias in the pattern of tree visitation during the MCMC search of the posterior tree distribution under model Ah. A few trees were visited at high frequency under this search (probably because they returned higher likelihood scores), but tree visitation was much more even across trees in the MCMC search under the other three models (and under all searches of the two-state character models).

## .Discussion

I found large differences in patterns of inferred ancestral character states and in the relative rates of character state change in different directions under different plausible models of character evolution. In general, the new Bayesian methods allowed me to account for topological uncertainty as part of the calculation of ancestral state probabilities, and to quantitatively compare alternative models to a null. In each of the four reconstructions, I found a best model of freely-estimated rates of character state change (Af) that was significantly different from the null (N) or I could not reject the null model in favour

of any alternative, including the heuristic alternative model (Ah) that incorporates ideas about homologies among modes of larval development in asterinid sea stars and in many other marine animal clades. However, the probability of the ancestral states inferred under these best models was generally much lower than the probabilities of ancestral states inferred under the heuristic alternative. In most cases, the best model and the heuristic alternative inferred different ancestral states (with a few notable exceptions such as lecithotrophy in *Meridiastra*; Figure 6.5). In principle, the availability of more taxa, additional life history information, and new analytical methods for ancestral state reconstructions might have helped resolve many of these ambiguities, which were apparent in earlier analyses by Hart et al. (1997), Cunningham (1999), and Huelsenbeck et al. (2003). All of these earlier analyses clearly indicated that the high rate of evolution of these reproductive characters among asterinid species, coupled with the close relationships and short branch lengths among asterinid clades, led to inferred ancestral states for these characters that strongly conflicted with simple ideas such as the homology of planktotrophic larval phenotypes among distantly related asterinid species with this mode of development.

In practice, the application of these new data and methods has not significantly resolved any of these difficulties for asterinids. I found, like others before me, that the model of character evolution is critically important both for the identity of the inferred ancestral state at most nodes and for the estimated probability of that state. I illustrated this issue (Figs. 6.2-6.5) by modeling

ancestral states under two different approaches: one that assumes a model of character evolution (Ah) based on general notions of homology among modes of larval development; and another that infers a model of character evolution from among several possibilities based on likelihood ratio tests and empirical estimates of rates of character state change. In almost all cases, these two approaches led to different inferences of ancestral character states. The most significant conclusion from our study is that (as before) the underlying model of character evolution is crucially important for ancestral state reconstruction.

The contrast between these two approaches highlights an important difference in the interpretation of ancestral reconstruction methods: do these methods infer the ancestral condition at a particular node in the tree of life (with more or less statistical confidence)? Or do these methods merely give insight into consequences of different assumptions about the order, rate, and reversibility of state changes (Strathmann 1978; Strathmann and Eernisse 1994)? The new methods of Pagel and Meade can be used as part of either approach, and it seems important for future users of these methods to state clearly which way they are being applied.

Several recent studies have implicitly used these methods in an exploratory mode to infer ancestral character states at nodes in a phylogeny. In some cases, models of discrete character changes have been treated as hypotheses, with an exploration of the implications of alternative models of character state change followed by further analyses (such as reconstruction of states at nodes, or analysis of correlated evolution of characters; e.g., Summers

et al. 2006). In many of these studies, as suggested by Mooers and Schluter (1999), the general one- and two-rate models of character change are tested against each other (Pereria and Baker 2005, Chapman et al. 2006, Vanderpoorten and Goffinet 2006). Studies that test the one- and two-rate models of character evolution often employ maximum likelihood methods to analyze ancestral character states on a most likely tree or a Bayesian sample of trees. Many studies, like ours, examine the implications of different hypotheses of the model of character evolution such as irreversibility, and often use likelihood ratio or Bayes factor tests to identify the most probable model (Collin and Cipriani 2003; Adamowicz and Sacherova 2006; Kohlsdorf and Wagner 2006; see Pagel 2004). Those studies that have recently used the Bayesian method of ancestral character state reconstruction can also use the properties of the posterior distribution in order to select the best model of character evolution, or can use it as an alternative model of character evolution when testing a hypothesis. Studies using the posterior distribution to choose the model have noted that the method of using MCMC importance sampling to model character evolution involves an additional assumption about the model of character evolution (Huelsenbeck et al. 2003; Pereira and Baker 2005; Moczek et al. 2006; Vanderpoorten and Goffinet 2006). This method is particularly attractive because it allows the estimation of character states at particular nodes, rates of character change between states, and the association between these rates and the tip states on the phylogeny (Nosil and Mooers 2005).

Fewer studies have critically examined the assumptions implicit in using these new methods in an exploratory mode to infer ancestral states, or advocated the explicit use of these methods only for comparing alternative models of character state change and the consequences of making different assumptions about the rate and reversibility of state changes (Pagel 2004). In one notable example, Igic et al. (2006) used ancient polymorphisms at the SI (self-incompatibility) locus shared among species of solanaceous plants to specify the ancestral presence or absence of self-incompatible mating systems at nodes in a species phylogeny. They showed that inclusion of such independent information about the history of mating system differences (that is, the independent *a priori* specification of a reliable model of the evolution of self-incompatibility) led to the conclusion that self-incompatibility is plesiomorphic for Solanaceae and irreversibly lost among some solanaceous clades. In contrast, a model of character evolution inferred *a posteriori* by optimization of the tip distribution of self-incompatibility onto the species phylogeny (without additional information from SI ancestral polymorphisms) in Bayestraits strongly suggested the parallel evolution of self-incompatibility among several solanaceous clades. Based on this observation, and the strong probability that shared ancestral SI allelic polymorphisms among species are direct and unambiguous indicators of self-incompatible mating systems in their common ancestors (a molecular fossil record of the mating system at these nodes), Igic et al. (2006) concluded that "The existing reconstruction methods, widely used in evolutionary analyses, may

sometimes fail to perform adequately when not informed by additional data" (see also Strathmann and Eernisse 1994; Pagel 2004; Finarelli and Flynn 2006).

Our results and those noted above strongly suggest that the use of quantitative methods of ancestral state reconstruction for inferring both the best-fit model parameters and the most probable ancestral states of developmental characters at nodes in the tree of life (rather than merely testing alternative assumptions about the evolution of those characters) requires models of character evolution constructed from first principles: genetic, embryological, ultra structural, physiological, behavioural, or paleontological data used to build a quantitative argument for homology among developmental character states (such as larval planktotrophy). A recent striking example of this approach uses embryological information from the evolution and development of nonfeeding gastropod larvae. Collin et al. (2007) showed that the phylogenetic distribution and maximum likelihood reconstruction of planktotrophy and benthic encapsulated development in *Crepipatella* limpets is consistent with the recent evolution of planktotrophy in *C. fecunda* from an ancestor in which encapsulated development was supported by ingestion of nurse eggs (within the capsule) instead of phytoplankton. This conventional interpretation based on the tip distribution of extant states and quantitative ancestral state reconstruction would be susceptible to the same problems of model development noted above for asterinids. However, the encapsulated veliger larvae of *Crepipatella* sp. (Collin et al. 2007) and *C. dilata* (Chaparro et al. 2002) retain most of the functional features (compound ciliary bands, food groove, velum) used for feeding by

planktotrophic veligers of *C. fecunda*. Collin et al. (2007) argue convincingly that the retention of the ancestral feeding morphology among some *Crepipatella* lineages with nonplanktotrophic life histories can be reliably used to identify specific cases of probable reversal to planktotrophy. Thus, a comparative phylogenetic model of similar probabilities for gain and loss of planktotrophy in *Crepipatella* can be supported by additional independent evidence from comparative embryology.

Quantitative estimates of the probability of homology among, for example, distantly related planktotrophic asterinids (*Asterina stellifera, Meridiastra mortenseni, Patiria miniata, Patiriella regularis*) could be substituted in Pagel's method for the qi→j values that are estimated empirically from the tip distribution of character states and the underlying tree topology and branch lengths. Unlike *Crepipatella* limpets, nonplanktotrophic asterinid embryos and larvae do not retain visible functional morphological remnants of their planktotrophic ancestry that could be used as guides. What other kinds of phenotypic data from asterinids, analogous to shared ancestral SI allelic polymorphisms in Solanaceae, could be used to build such models?

Recent comparative studies have revealed two significant differences between lecithotrophic and planktotrophic asterinids (other than the obvious morphological differences associated with feeding). First, large eggs of lecithotrophs have a high concentration of wax esters and other lipids, whereas planktotrophic species develop from eggs with a major yolk protein (and little lipid) as the principal source of organic energy for early development (Villinski et

al. 2002). Second, lecithotrophs show highly modified patterns of early cleavage division relative to the holoblastic cell divisions of planktotrophs (Cerra and Byrne 2004). If the loss of planktotrophy is reversible and some planktotrophic asterinids (such as Meridiastra mortenseni) have evolved this mode of development from a recent lecithotrophic ancestor, then some planktotrophs might be expected to have higher egg lipid concentrations and vestiges of meroblastic or syncytial cleavage divisions (as a holdover from the recent ancestral condition) in comparison to the eggs of asterinids with planktotrophic larvae as a plesiomorphy (such as *Patiria* species). Such vestigial features might be a common and rich source of information for models of evolution of marine larvae (e.g., Kusakabe et al. 1996).

The relative abundance of egg lipids or the number of modified cleavage divisions in planktotrophs could be used as part of a quantitative model (along with other data) of the probability of homology of this character state among asterinids. The development of such quantitative estimates of homology among larval forms could provide a theoretical framework for this branch of evolutionary developmental biology (Strathmann and Eernisse 1994; Serb and Oakley 2005; Dunn et al. 2007). However, until such models are available, the quantitative reconstruction of ancestral states for developmental characters should be carried out in the spirit of hypothesis testing rather than of the inference of ancestors.

## .General Conclusions

I this chapter I find that variation in the model of character evolution can severely bias results of ancestral character state reconstruction in the Asterinidae. Despite the advent of recent methods, which more thoroughly search parameter space for the most probable model of character evolution and the most probable ancestral character state, I was unable to conclusively infer ancestral characters throughout the asterinid phylogeny. The diversity of character among the tips of the asterinid phylogeny suggests that life history characters evolve rapidly in the Asterinidae and there may be a high probability of parallelisms among groups. However it also implies that the level of phylogenetic constraints on the evolution of life history may be minimal. The low likelihood of accurately reconstructing ancestral character states in the asterinids suggests that analysis such as phylgoenetically independent contrasts may fail to produce results that are robust to the model of character evolution I use in my analysis. I will proceed with the comparative analysis in light of these results in an attempt to analyse the correlated evolution of life history and population genetic structure. I will use methods that emphasize the comparison of character states among tips instead of potentially biasing my results by inaccurately plotting the evolution of characters through the phylogeny.

**Figure 6.1 Path diagrams summarizing the results of Bayesian inference and comparison of a null model (**N**, heavy line in the central box of each panel) and three alternative models of evolution for four developmental characters in which character states are freely evolving (**Af**), constrained by the heuristic model (**Ah**), or constrained in the reverse direction (**Ar**) . For each character, states are coded 0-1 or 0-3 as in the text. Numbers in italics are ln(likelihood) scores; models that better fit the data and phylogeny have less negative scores. Three model comparisons (arrows) and their associated Bayes factors (*Bf*) are shown for each character. An alternative model that was a significantly better fit than the null model (N) has *Bf* > 2.5 (in bold). The best-fit model (N in panels A, D; Af in panels B, C) is indicated by grey shading; ancestral character state reconstructions are shown for that best-fit model and for model Ah in Figures 6.2 – 6.5. Rates of character state change ($q_{i \to j}$) are also shown for those two models.**

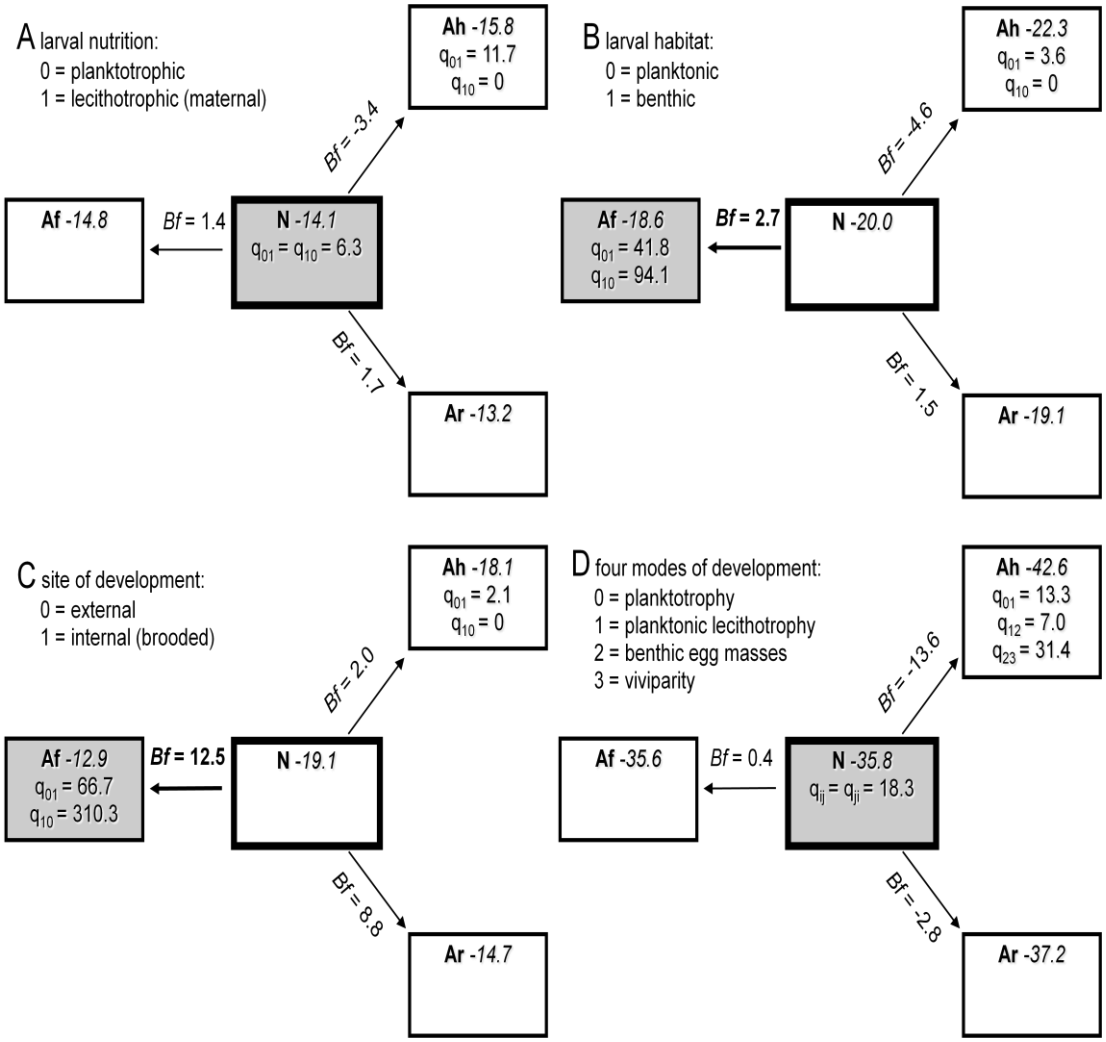


**Figure 6.2 Probabilities of planktotrophy (0) or some maternal source of nutrition (lecithotrophy; 1) reconstructed as ancestral states mapped onto 1000 trees from the posterior distribution of topologies and branch lengths in a Bayesian analysis of mtDNA sequences from 28 asterinid species. The topology shown is the 50% majority rule consensus of those 1000 trees. Branch lengths are not drawn to scale. Character states for extant taxa are shown between terminal branch tips and taxon names. Two reconstructions are shown for each node: one above the corresponding branch based on the most probable model of character evolution (model N; rates as in Figure 6.1A); and one below the corresponding branch based on the heuristic alternative (model Ah). Integers (0 or 1) to the left of each colon indicate the most probable ancestral character state; proportions to the right of each colon indicate the probability of that character state.**

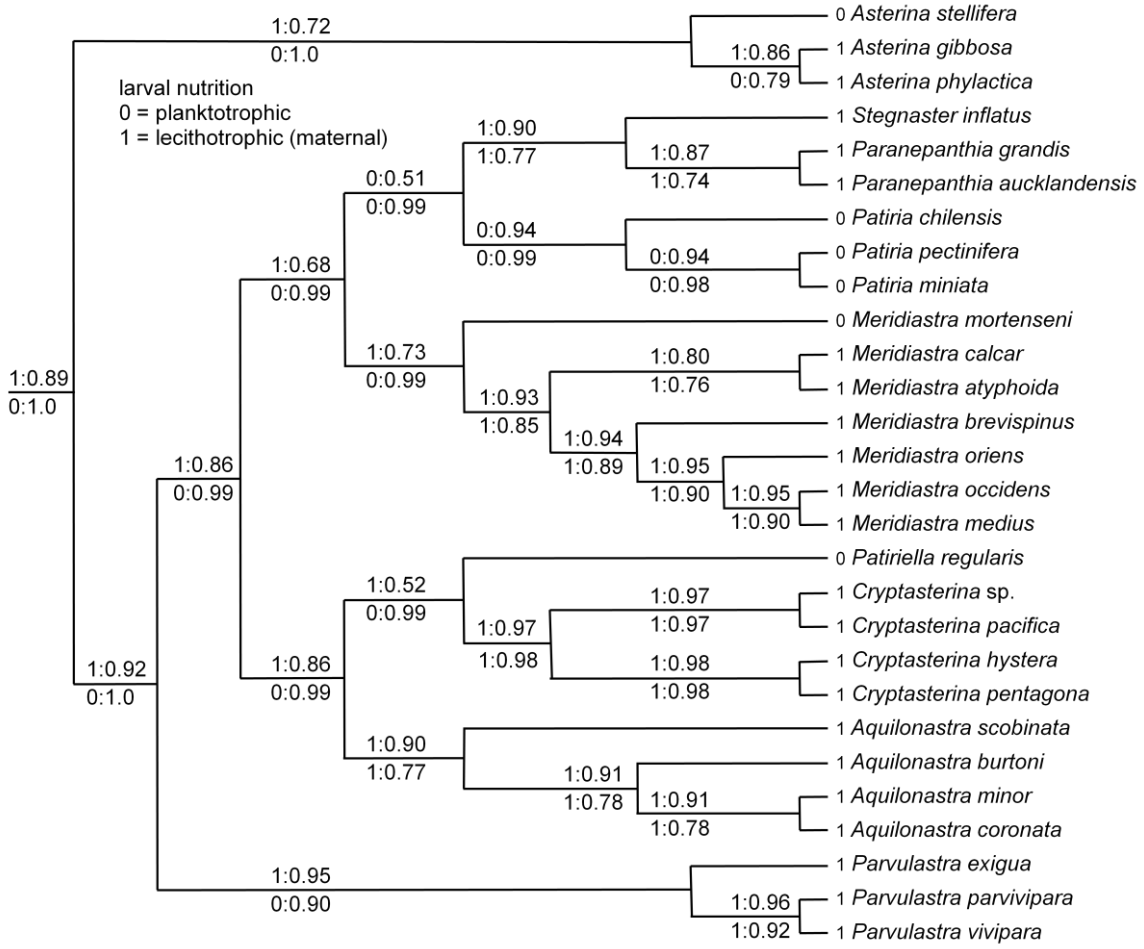


**Figure 6.3 Probabilities of planktonic (0) or benthic (1) larval development as ancestral states (as in Fig. 2). Two reconstructions are shown for each node: one above the corresponding branch based on the most probable model of character evolution (model Af; rates as in Fig. 1B); and one below the corresponding branch based on the heuristic alternative (model Ah).**

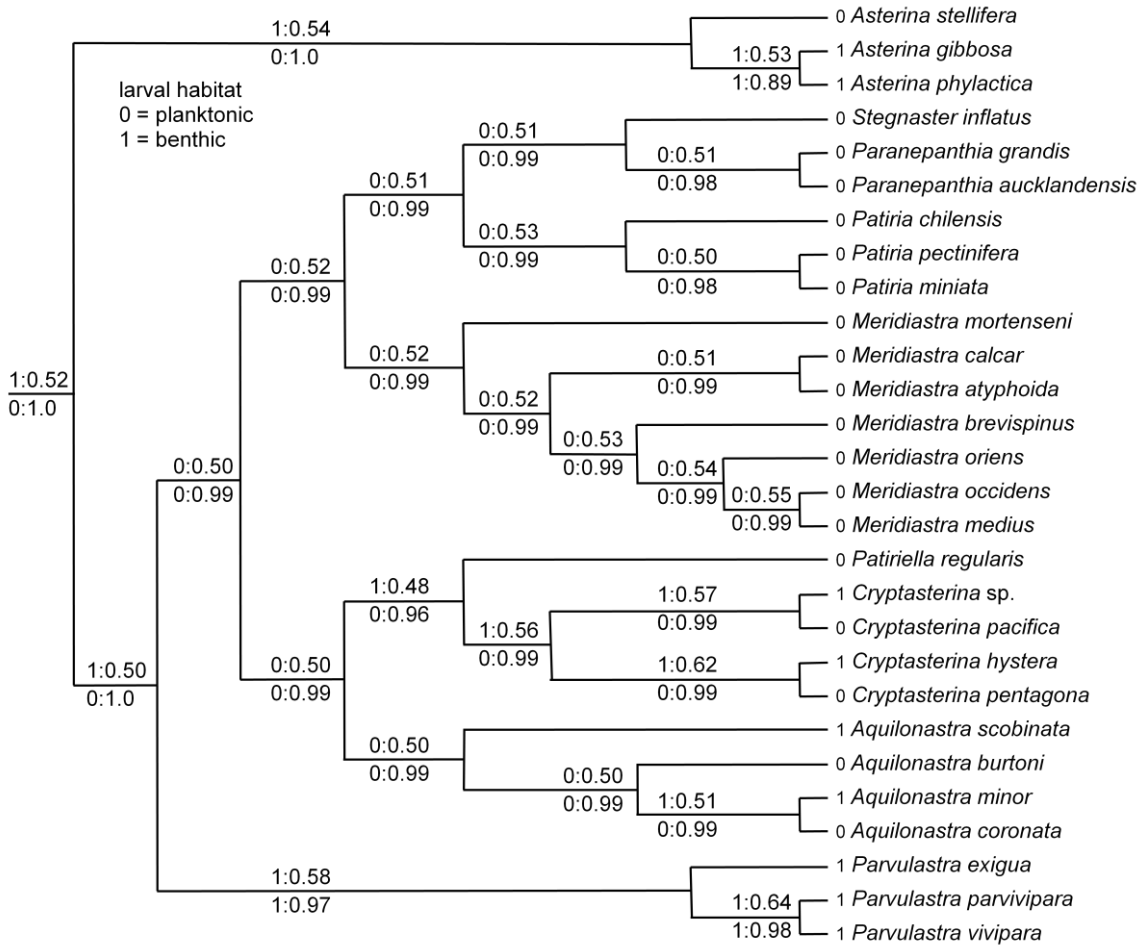


**Figure 6.4 Probabilities of external (0) or internal (brooded; 1) larval development as ancestral states (as in Fig. 2). Models and rates as in Fig. 3.**

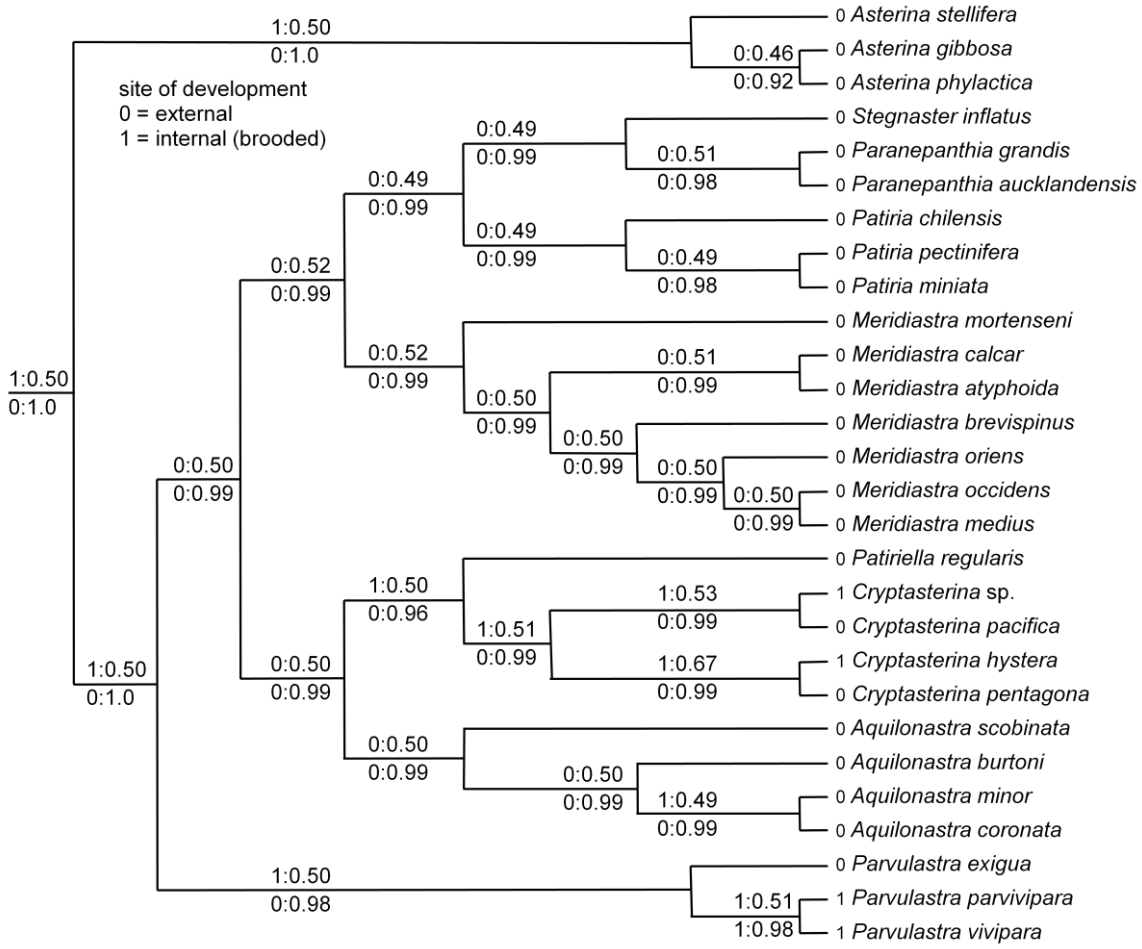
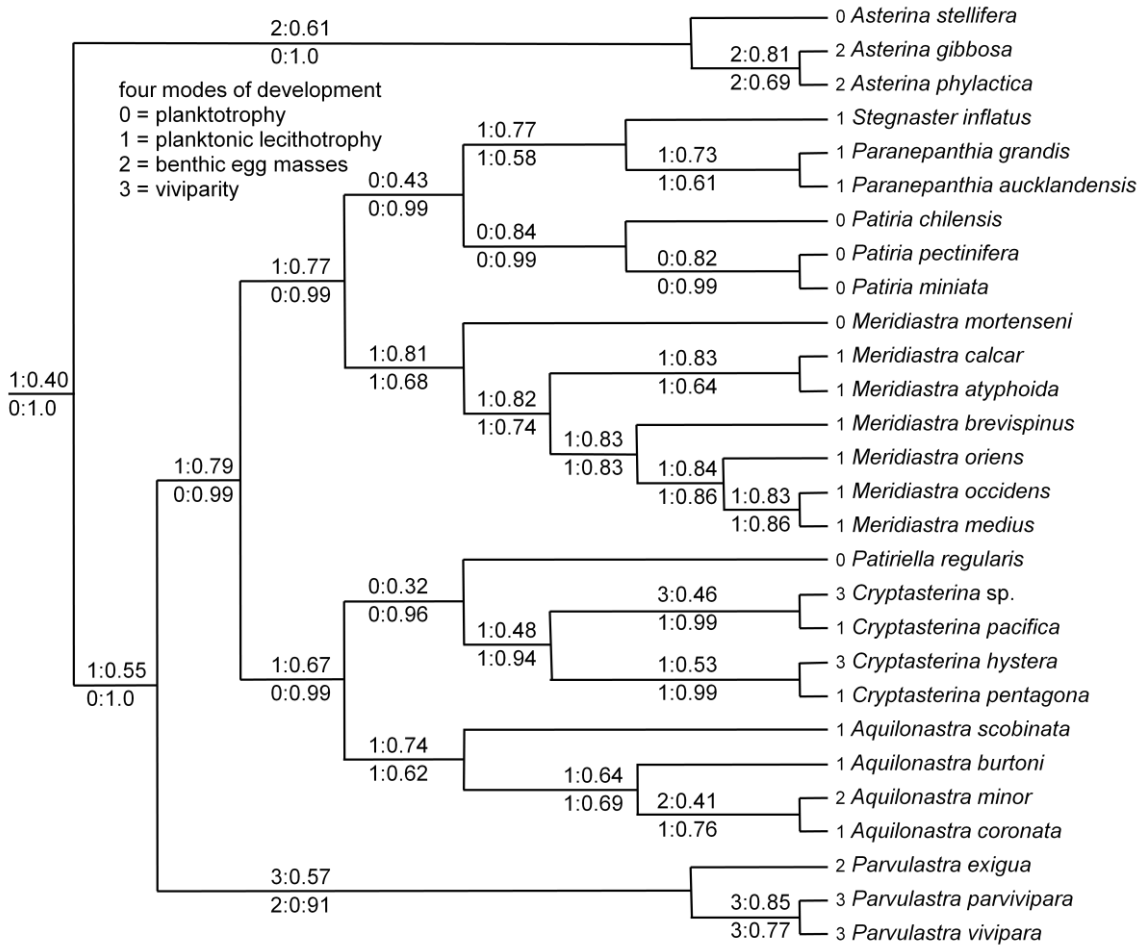


**Figure 6.5 Probabilities of planktotrophy (0), planktonic lecithotrophy (1), development in benthic egg masses (2), or viviparous brooding (3) as ancestral states (as in Fig. 2). Two reconstructions are shown for each node: one above the corresponding branch based on the most probable model of character evolution (model N; Fig. 1A); and one below the corresponding branch based on the heuristic alternative (model Ah).**



# Chapter 7 **COMPARATIVE ANALYSIS OF THE RELATIVE CONTRIBUTION OF LIFE HISTORY TO POPULATION GENETIC STRUCTURE**

## .General introduction

The role of life history in population genetic structure is a fundamental question in biology, especially in disciplines such as ecology, evolution, and conservation, where changes in population genetic variation influence population dynamics such as migration, growth, decline and speciation (Kinlan and Gaines 2003). Here I examine the relative role of life history in shaping population genetic variation in asterinid sea stars where similar life histories have evolved multiple times in parallel. My comparative analysis is conducted using a linear model that controls for phylogenetic non-independence among organisms. I find that levels of within- and between-population genetic variation are highly correlated with some life history characters, and that the degree to which phylogenetic covariance among species should be incorporated into the model varies among comparisons.

## .Introduction

A primary focus of the study of biology is in finding correlations between phenotypic characters among species that may inform predictions about general processes in nature (Pagel 1999b). Comparative analyses of phenotypic evolution often emphasize the importance of controlling for phylogenetic non-independence among taxa while simultaneously cautioning against the failure to appreciate the underlying assumptions of comparative methods (Felsenstein 1985, Freckelton 2007). Nevertheless, by taking potential historical constraints of character evolution into account these methods can more precisely predict the correlated evolution of phenotypic characters (Garland and Ives 2000, Harvey and Rambaut 2000, Freckelton et al. 2002, Rohlf 2006). Accounting for historical or phylogenetic effects is complex because the degree to which phylogeny affects parameter estimates may vary among taxa and characters and subclades, and because methods for comparing phenotypic characters among organisms are numerous and diverse (e.g. Harvey and Pagel 1991; Martins 1996; Oakley and Cunningham 2000; Paradis 2006).

Felsenstein (1985) first suggested a method that uses phylgoenetically independent contrasts (PIC) to control for phylogenetic non-independence (see also Martins 1996, Martins and Hansen 1997, Paradis 2006). A second method first proposed by Grafen (1989) and expanded by Martins and Hansen (1997) and Freckelton et al. (2002) corrects for non-independence by incorporating phylogenetic relationships of the data points into the error of a linear model, using a Generalized Least Squares. This method incorporates the variance due to

phylogenetic signal among characters using a correlation structure that is built into a variance/covariance matrix among characters in a maximum likelihood optimization framework. In general the defined correlation structure predicts co-variance in character states among closely related species by a variable proportional to their phylogenetic relationship. Paradis and Claude (2002) expanded on a similar method developed by Liang and Zeger (1986) of generalized estimating equations, which correct the residual variation among characters based on phylogenetic relatedness using a correlation matrix in a linear model. This method allows for the response variable in a linear regression to be non-normally distributed, which is especially useful with discrete response variables (Garcia 2007). One difficulty of the GEE framework is that it is not optimized by maximum likelihood, and it is therefore difficult to compare the overall fit of two models with contrasting parameterization.

Recent studies of the relative influence of life history on population genetic structure in benthic marine species have rarely used methods to incorporate phylogenetic relationships among organisms or have explicitly justified not using such methods. Some studies comparing a broad taxonomic array of species have used a taxonomic or phylogenetic correction of the linear model in their approach (Bohonak 1999, Bradbury et al. 2008, also see Garcia et al. 2009, Kelly and Palumbi 2009) while other studies comparing species from closely related evolutionary assemblages use sister species comparisons thus controlling for similarities arising due to shared ancestry (Kyle and Boulding 2000, Dawson 2002, Hickerson and Cunningham 2005). However, the approach of comparing

sister species requires large numbers of species pairs, uses little of the information on trait covariation among clades other than sister species, and breaks down when quantitative methods are applied to comparisons of more than two polyphyletic species (Garland and Adolph 1994). Finally many analyses examining a broad array of distantly related species lack a reliable estimate of the phylogenetic relationships among organisms and compare species directly without accounting for phylogenetic non-independence in the analysis (Teske et al. 2007, Weirsing and Toonen 2009, Ayre et al. 2009)

In this study, I examine sea stars from the family Asterinidae that have similar modes of development and mating systems that have evolved multiple times in parallel. I use microsatellite analysis of population genetic variation to assess the relative influence of life history on population genetic structure in asterinid sea stars. I conducted a comparative analysis of nine species from the family Asterinidae, examining the relationship between life history and population genetic characters using a Generalized Least Squares (GLS). I compared GLS models with and without control for phylogenetic non-independence and I assess model fit when phylogenetic signal is incorporated into, or left out of the GLS.

If life history contributes significantly to genetic variation then species with planktonic larva or gonochoric mating systems should exhibit low levels of population genetic differentiation. In contrast, species with larval forms that lack planktonic dispersal or are hermaphroditic will have higher levels of genetic differentiation and lower allelic richness due to a greater probability of inbreeding leading to loss of alleles via genetic drift. These characters (planktonic vs.

benthic and hermaphroditic vs. gonochoric) are highly correlated with a suite of characters related to fitness of a given larval life history and include, egg size, body size, bathymetric depth of habitat, reproductive season, habitat type and mode of larval nutrition (Emlet 1987, Emlet 1995). Each of these life history traits can be used as representative characters for asterinid life history strategies, as they are similarly predicted to be related to patterns of population genetic variation. Alternatively, a lack of relationship between life history traits and population structure would indicate that life history characters such as larval development and mating system have little influence on genetic variation and that alternative explanations based on historical and contemporary environmental factors or phylogenetic inertia are more important for shaping population structure in the Asterinidae.

## .Materials and Methods

Most comparative methods, including the GLS method used in this analysis, are most suitable for continuous response and predictor variables, while some life history variables such as larval form and mating system vary as suites of categorical variables whose differences are expected to predict population genetic structure. In this analysis I have used three methods to characterize modes of development. First, I coded the mode of development as a continuously distributed numerical variable for analysis with continuously distributed population genetic parameters ($F_{st}$, allelic richness, heterozygosity). Second, I code the mode of development as a four state variable ordered by dispersal potential. Third, I used a suite of dummy variables to code life history

characters into two-state and four-state independent categorical variables (Stevens 2002).

Discrete life history characters (LH) were classified as planktonic feeding larvae with dioeceous adults (1), planktonic non-feeding larvae with dioeceous adults (2), benthic egg mass development with hermaphroditic adults (3), and live bearing with hermaphroditic adults (4). These variable values (1-4) are ordered by dispersal potential from highest (1) to lowest (4). This approach assumes that planktotrophic species (1) have twice the dispersal potential of planktonic lecithotrophs (2) (and 3 times the dispersal potential of species in benthic egg masses, etc.).

In addition to life history differences characterized as modes of development (1-4 above), I also analyzed covariation between population genetic variables and continuously distributed life history variables including egg diameter and adult body size. Data for egg diameter (ED) was collected from Byrne 2006. For the comparative analysis, egg size data were transformed by the natural logarithm to improve normality.

Data for adult body size (AR) were collected from Prestedge 1998, O'Loughlin et al. 2002, O'Loughlin and Waters 2004, and Byrne et al 2006. All estimates were based on maximum arm radius or diameter. For some species size was expressed as the distance from the centre of the arm to the arm tip. Arm radius data was transformed by the natural logarithm to improve normality.

Finally, I transformed the four state discrete character into a suite of 3 dummy variables coded as 0 and 1. These dummy variables turn the discrete

data into a set of testable covariates of categorical effects based on transitions within the four state character such that dummy 1 (D1) is 0=feeding 1=non-feeding, dummy 2 (D2) is 0=planktonic 1=benthic, dummy 3 (D3) is 0=external development 1=internal development. This method of coding the data often improves model fit but can be problematic in studies with few data points (such as this one), as the degrees of freedom are decreased. For this reason, I also ran the analysis with life history strategies divided into two state characters coded as one dummy variable (rather than 3). The first two-state character is the presence or absence of feeding larvae (0=feeding and 1=non-feeding) (FN) and the second the presence or absence of a planktonic larvae (0=planktonic, 1=benthic) (PB). The third two state dummy variable represents site of development (0 = external development, 1 = internal development).

**Population Genetic Analysis**

I use population genetic data on total allelic richness (Rch), heterozygosity (Het) and global $F_{st}$ from Chapters 3 and 4 of this thesis to carry out the comparative analysis. Data measured as proportions such as $F_{st}$ and Heterozygosity were transformed using the inverse logistic or 'logit' transformation for the best improvement on normality, while allelic richness data was transformed by the natural logarithm to improve normality. For the live bearing species *Cryptasterina hystera* global $F_{st}$ and Heterozygosity values were calculated at 1 and 0 respectively. These values have a 'logit' transformation

with no solution and therefore were remapped to 0.9999999 and 0.0000001 respectively. Changes in the degree of remapping had no bearing on the results.

**Phylogeny Estimation**

I used a maximum *a posteriori* tree from the analyses in Chapter 6 to generate a pruned tree for my comparative analysis. Since data did not conform to a molecular clock the branch lengths on the full tree were first transformed using the non-parametric rate-smoothing algorithm in R (Sanderson 1997), creating an ultrametric tree. The tree was pruned using the drop.tips command in R to incorporate only the nine species for which I have life history and population genetic data (Figure 1).

**Tests for Phylogenetic Signal and Correlations Among Characters**

All comparative analyses and tests for phylogenetic signal were conducted using the software package R (R Development Core Team 2005, www.R-project.org). One of the major conclusions of Chapter 6 of this thesis was that life history characters evolve rapidly along the asterinid phylogeny and thus may not show a high degree of phylogenetic inertia (signal) in their evolution. Because of this, the degree to which I should control for phylogenetic non-independence in comparative analysis of population genetic structure and life history is less certain and may vary among life history characters and among-population genetic variables (Losos 1999, Harvey and Rambaut 2000). Similarly, while many researchers advocate the use of a phylogenetic correction in the

comparative method they also recommend testing for phylogenetic signal before the appropriate corrections are made (Freckleton et al. 2002).

First, I tested for phylogenetic signal of each life history character and population genetic statistic independently using Moran's index (I) (Gittleman and Kot 1990). This parameter estimates the autocorrelation among characters along a phylogenetic tree. Significance was tested with 1000 permutations of the data.

I examined the relationship between life history and population genetic parameters using the Generalized Least Squares (GLS). I used the method of Pagel (1999) to detect the presence of phylogenetic signal in the GLS framework. I estimated the relationship between life history and population genetic parameters assuming no phylogenetic signal among characters and then incorporating some degree of phylogenetic signal into the GLS model.

The method of Pagel (1999) uses the value lambda (λ) (a measure of phylogenetic signal) in the covariance matrix of a GLS model to incorporate a correlation structure between two characters (Freckleton et al. 2002). Lambda can be optimized in a maximum likelihood framework or fixed to a value of interest to the researcher. A lambda no different from 0 indicates no phylogenetic signal in the phenotypic characters while a lambda value greater than zero indicates that there is some degree of phylogenetic signal in the relationship between characters.

For each pair of life history variables and population genetic parameters I ran a Generalized Least Squares (GLS) model with the correlation structure set to the maximum likelihood value of lambda inferred from the model. For two

comparisons, allelic richness by four state discrete life history (LH) and Fst by the two state planktonic benthic (PB) character, the GLS run estimating lambda failed to converge on a maximum likelihood value, and as such for this comparison I conducted an additional run for lambda fixed between 0 and 1 at 0.5 to simulate an intermediate level of phylogenetic signal.

As an exploratory analysis, I conducted a second set of analyses for each pair of life history variables and population genetic parameters using a GLS model with the correlation structure set to a lambda of zero. This model assumes that there is no phylogenetic signal in the correlations among characters.

I used AIC values generated from models to calculate $AIC_c$ values, which penalize likelihood estimates for small sample sizes. $AIC_c$ values for models where lambda was set to zero and where the maximum likelihood value of lambda was inferred were compared to one another for each pair of life history variables and population genetic parameters. A difference of greater than two points between compared $AIC_c$ values was taken as sufficient to consider the model with the smaller $AIC_c$ value to be a better fit (Burnham and Anderson 2004).

**Graphical Representation of the Comparative Data**

To display the influence of phylogeny vs. dispersal potential on each of the three population genetic parameters ($F_{st,}$ Allelic Richness and Heterozygosity) I produced bar plots of each of the population genetic statistics first ordered by dispersal mode and then by phylogenetic relationship. In each of the graphs of

population genetic structure by life history trait, plots were ordered from highest to lowest dispersal potential.

## .Results

### Phylogeny Estimation, Character Coding and Population Genetic Data

The phylogeny was estimated from the maximum aposteriori phylogenetic tree in chapter 6 (Figure 7.1). Branch lengths deviated from clocklike evolution and thus were made to conform to a molecular clock by non-parametric rate smoothing (Sanderson 1997).

The continuous and discrete life history characters as well as 4 state dummy variables, and discrete 4 state life history codes used in this analysis can be found in table 7.1.

Population genetic parameters used in this analysis can be found in Appendices 4 and 5 (heterozygosity and allelic richness) and in Table 7.2. The global *F*st were standardized to a maximum value of 1 and these were taken from the non-structured AMOVA analysis of between population genetic variation for each species (Table 4.2).

### Tests for Phylogenetic Signal and Correlations Among Characters

The Moran's index test of phylogenetic autocorrelation showed that none of the life history characters or population genetic parameters showed any degree of phylogenetic signal (P = 0.75 – 0.99).

Results of the linear models of the relationship between life history and population genetic structure are given in Table 7.3. These comparisons are modelled in two different ways, however the basic comparisons remain the same and as such, the standard notation for the comparison between a life history variable and a population genetic statistic is as follows: population genetic statistic ~ life history variable. I tested two models of the relationship among-population genetic and life history characters, which included and Generalized Least Squares with the lambda value of the correlation structure set to zero and another GLS model with the lambda value inferred from the model. I recorded the slope, standard error, lambda value (when appropriate), log likelihood value and $AIC_c$ value in Table 7.3. A test for model fit was conducted using $AIC_c$ values. Models with $AIC_c$ values superior to their competing models by a value of 2 or more are highlighted with grey bars.

Since tests of phylogenetic signal using Moran's index suggested life history and population genetic characters lacked a significant level of phylogenetic signal, I suspected that in many cases a model with no phylogenetic signal would be the best fit to the data. Indeed, in some cases the best model was one with no phylogenetic co-variation ($\lambda = 0$), ($F_{st}$~LH: $AIC_c = 59.29$, Het~LH: $AIC_c = 63.57$ $F_{st}$~AR: $AIC_c = 60.31$, Rch~AR: $AIC_c = 20.66$, Het~AR: $AIC_c = 65.21$, $F_{st}$~ED: $AIC_c = 57.18$, Rch~D: $AIC_c = 12.85$, $F_{st}$~FN: $AIC_c = 58.42$, Het~FN: $AIC_c = 63.36$ and Rch~PB: AIC = 14.67). In only three of these cases the relationships between variables was significant (Rch~AR: P = 0.0001, Rch~D2: P = 0.03, Rch~PB: P = 0.001). Furthermore, in some cases the

superior fit of the model assuming no phylogenetic signal (λ = 0) could be predicted by the small optimized maximum likelihood lambda value (λ = ML) in Table 7.3 (egg. $F_{st}$~AR, Rch~AR), while in other cases the lambda values are larger and do not predict that the GLS model with lambda equal to zero might be the best fit ($F_{st}$~LH, $F_{st}$~FN, Het~LH).

I incorporated phylogenetic signal into tests of the relationship between life history and population genetic variation using the GLS with the degree of phylogenetic signal specified by lambda (λ). Maximum likelihood values of lambda ranged from (λ = -2.6 to 1.4) with a mean value of 0.29. Note that lambda can assume values greater than one and less than 0 because the optimization process for lambda finds the best transformation of the phylogenetic correlation structure that makes the trait data best fit Brownian motion (Freckleton et al. 2002). For example, a lambda value greater than one can arise if the values of closely related species are more similar than predicted by the Brownian motion process.

Many of the relationships between life history variables and population genetic parameters were significant or marginally significant under a model that controls for phylogenetic non-independence by incorporating lambda into the correlation structure ($F_{st}$ ~LH: P = 0.03, Rch~LH: P = 0.000, Het~LH: P = 0.001, Rch~AR: P = 0.000, $F_{st}$~ED: P = 0.03, Rch~D2: P = 0.02, Rch~FN: P = 0.002, $F_{st}$ ~PB: P = 0.01, Rch~PB: P = 0.001, and Het~PB: P = 0.000). However there were only three cases in which the model of phylogenetic signal predicted by lambda was the best fit for the data ($F_{st}$~D: $AIC_c$ = 46.86 and Het~D: $AIC_c$ =

43.86, and Het~PB: $AIC_c$ = 34.04), and only one of these (Het~PB) had a significant slope.

### Graphical Representation

Finally, I plotted population genetic parameters on the y-axis against species with decreasing dispersal potential on the x-axis and found a qualitative relationship between population genetic variation ($F_{st}$, Allelic Richness and Heterozygosity) and dispersal potential. When species were plotted on the x-axis against phylogenetic relationship I found that the population genetic parameters were often more likely to be different among sister species than among species with similar life histories (and thus dispersal modes) found in different clades (Figure 7.2).

## .Discussion

Overall this study has shown that in many cases life history characters can predict levels of population genetic structure in asterinid sea stars. In most instances these results were consistent with and without controlling for phylogenetic non-independence in the linear model. However the degree to which incorporating phylogenetic signal into the model changed or improved model fit varied among comparisons.

## Correlations Among Characters

Life history (LH) as a four state discrete character was significantly correlated with $F_{st}$, heterozygosity (Het) ,and allelic richness (Rch) for the GLS model that incorporated the maximum likelihood value of lambda. However, only allelic richness and life history were significantly related for the GLS model that set lambda equal to zero. In the relationship between Life history (LH) and $F_{st}$ and Heterozygosity, the best-fit model was that where lambda was fixed at zero, while in the relationships between allelic richness and life history, incorporating phylogeny had little bearing on the fit.

Arm radius as a proxy for the four-sate life history mode was only significantly related to allelic richness, but regardless of whether phylogeny was explicitly included in the model. Indeed, the GLS model with lambda set to zero was a better fit to this data for all three comparisons of arm radius and population genetic parameters.

The relationship between continuous proxy egg size and population genetic variation produced weaker patterns than the life history parameters mentioned above. The among-population component of genetic variation ($F_{st}$) was the only population genetic parameter that was significantly related to egg size and only for the GLS model where the lambda value was inferred. This result is somewhat surprising as egg size as well as body size is usually highly correlated using the 4 state life history mode (such as feeding vs. non-feeding larvae or internal vs. external fertilization) in asterinids and other benthic marine invertebrate taxa (Strathmann and Strathmann 1982, Villinski et al. 2002, Byrne

2006). One interesting observation related to egg size in the Asterinidae that could have led to this distinct result, is that egg size in two live bearing species (*P. vivipara*, and *P. parvivipara*) is secondarily reduced, likely due to sibling brood cannibalism and the lack of a requirement of maternal provisioning of yolk nutrients in the egg (Byrne 1996, Prowse 2008). This exception would still permit egg size to be correlated with *F*st, because of the lack of between population genetic structure in *P. vivipara* likens it (in both egg size and $F_{st}$) to species with planktonic feeding larvae, which have smaller eggs. However, both *Parvulastra* species have low levels of allelic richness and heterozygosity relative to species with planktonic feeding larvae, and the secondarily reduced eggs of these species could confound the predicted relationship between these characters.

With few exceptions (i.e. Rch~LH, RCH~ED, and Het~ED) the GLS models for the four sate character and the continuous proxies (egg size and arm radius) were best fit by a model that fixed the lambda value at zero. This result is corroborated by the maximum likelihood value of lambda inferred by the linear model ($\lambda$= ML) in that many of the lambda estimates are small or negative. Two noticeable exceptions are Het~ED and $F_{st}$~LH, which both have lambda values larger than 0.4, making their inferred lack of phylogenetic signal detected when testing models equivocal (Table 7.3).

Dummy variables for the four state discrete life history characters did not show routinely significant relationships with population genetic structure. However, the variables D1 (representing the transition between feeding and non-

feeding larvae) and D2 (representing the transition between planktonic and benthic organisms) were significantly related to allelic richness. Both the relationship between D2 and D1 and allelic richness were best fit to the GLS model where lambda was fixed at zero.

The life history modes separated into two-state characters showed a variable degree of significance in their relationship with population genetic parameters. The life history trait that represents the transition between feeding and non-feeding was only significantly related to allelic richness (for both $\lambda = 0$ and $\lambda = ML$). The two-state life history trait that represents the transition between planktonic and benthic development was significantly related to allelic richness when the lambda value was fixed at zero and was significantly related to $F_{st}$ , allelic richness and heterozygosity when the lambda value was inferred by the model. Interestingly, the analysis of the relationship between Heterozygosity and planktonic vs. benthic development was the only comparison aside from the dummy variable analysis that was best fit by the model that inferred the maximum likelihood value of lambda. In this case lambda was the highest in the analysis at (1.43), suggesting a high degree of phylogenetic signal in the correlation among these characters (Table 7.3).

**Importance of Data Points and Phylogeny**

One noteworthy inconsistency in this data is the global *F*st value in *P. vivipara* relative to other live bearing species. *F*st (between population component of genetic variation) in this species was on a similar order to that of

species with planktonic feeding and non-feeding larva that are capable of long distance dispersal and a high degree of out-crossing.

Several potential explanations exist for this unusual pattern of among-population genetic variation in *P. vivipara* relative to other livebearers. First, the natural history of Tasmania has been wrought with extreme fluctuations in temperature, glacial cover, salinity, and sea level, which could have differentially impacted *P. vivipara* relative to other live-bearing species (Colhoun 1996, McGowran 1997, Colhoun 2000, Nuernberg et al. 2004, Waters 2008, Fraser et al. 2009). Secondly, *P. vivipara* has levels of within-population genetic variation, and estimates of effective population size similar to other live-bearing species and is therefore expected to have experienced similar levels of genetic drift relative to other live bearing species. However, genetic drift is a stochastic process and it is likely that alleles drift to fixation and extinction differently among populations and among species, which could ultimately lead to differences in the partitioning of genetic variation among populations in the three species. Finally, we also know that *P. vivipara has* a low census size and is listed as threatened (see Chapter 4). It is therefore not impossible that the similarities in allele frequencies among populations are the results of 'good Samaritans' moving *P, vivipara* individuals among populations.

This analysis demonstrates that the influence of phylogeny in the comparative method is inconsistent among suites of characters across the three population genetic parameters. In many cases including some level of phylogenetic signal in the analysis by estimating lambda significantly reduces the

standard error of the slope (e.g. $F_{st}$~ED, $F_{st}$~PB). In fewer cases the standard error is reduced when the lambda value is fixed at zero (e.g. $F_{st}$~LH) More importantly the degree to which one type of correlation structure provides a best fit to co-variation among life history and population genetic parameters was highly biased toward the GLS with lambda fixed at zero. One likely explanation for this result is that the $AIC_c$ values are penalized by the numbers of parameters in the model, such that a model with more parameters is penalized more heavily than a model with fewer parameters. In this comparative analysis I add an additional parameter when inferring lambda from the GLS, which penalizes the $AIC_c$ value of this model ($\lambda$ = ML) more heavily then the GLS with lambda fixed at zero ($\lambda$ = 0). As a result, it is not completely surprising that the model with fewer parameters was usually a better fit to the data. Despite the greater penalty for an additional parameter, the GLS model that estimates lambda is a better fit to the data in three cases ($F_{st}$~D, Het~D, and Het~PB) and in all cases the degree of phylogenetic signal measured as lambda is high (1.40, 1.41, and 1.43 respectively). In many cases (e.g. , Rch~LH) there was no best-fit model for the relationship between variables and the relationship varied in significance among models in these cases.

The outcomes of my GLS models are heavily dependent on the assumption that the residual values are normally distributed around the fitted line. Data were transformed to improve normality and indeed conform to a normal distribution. Nevertheless patterns of residual variation are biologically interesting, and further examination of residual variation may show patterns of

variation in population genetic structure unidentified by my analysis (Zuur et al. 2009).

One caveat of this study is that two species (*Meridiastra mortenseni* and *Patiria pectinifera*) were not thoroughly sampled, however, the lack of sampling was on different scales in the two species. In the case of *Meridiastra mortenseni,* I sampled only three populations that spanned much of the species total range. In the case of *P. pectinifera* I sampled three populations along a very narrow (16 km) band of coastline that represented only a small portion of this species' entire range. Further sampling of these species could reveal more complicated population genetic patterns similar to those of its congener *Patiria miniata. In P. miniata,* population genetic structure reflects the dispersal potential of the larval form in some regions while being influenced by historical vicariance in others (see Chapter 2). Similarly the sampled ranges of many species in this analysis varied. Some of the variation in sampling range reflected the actual range of each species (*P. vivipara*) while some were limited by sampling restrictions, time, and resources (*C. hystera*).

Sampling a greater number of species could also influence the relationship between life history and genetic structure. For example, if Atlantic asterinid species *Asterina,* which are the sister group to the Indo-Pacific clade of species in my study, are fundamentally different in the way life history influences population genetic structure then the sampling of this clade could present an alteration of the patterns I see in this analysis. In the intertidal sea star *Asterina gibbosa* the geography of populations (whether in the Atlantic or Mediterranean)

greatly influences the levels of between population genetic variation. In the Atlantic, population differentiation is consistent with isolation by distance, but the Mediterranean populations show overall very high levels of differentiation and high population-specific assignment of alleles (Baus et al. 2005). This study also showed that the greatest amount of genetic variance was explained by regional differentiation between the Mediterranean and Atlantic Oceans. The results suggest that Atlantic asterinids have complicated population genetic structure linked to several aspects of the species' intrinsic dispersal ability as well as external forces (Baus et al 2005).

**Significance**

This study is one of few to employ the generalized linear modelling framework to control for phylogenetic non-independence in the comparison between population genetic structure and life history (see also Duponchelle et al. 2008, Duminil et al. 2007, and Bradbury 2008). The phylogenetic comparative method has been deemed essential in many cases in order to incorporate the non-independence among data points into the comparative analysis (Felsenstein 1985, Pagel 1999). However several authors have suggested that the degree of phylogenetic signal among characters is an important consideration when beginning to analyze comparative data (Freckleton et al. 2002). In my analysis there appears to be a variable level of phylogenetic signal, and significance depending on the characters examined, suggesting that accounting for

phylogenetic non-independence is important in some cases and less important in others.

This study builds upon data from comparative analyses addressing the relationship between life history and population genetic structure in both broad taxonomic samples as well as closely related species (Bohonak 1999, Weirsing and Toonen 2008, Bradbury et al. 2008, Marko et al. 2009). Since life history variation is an important predictor of a species distribution, migratory potential, and ecological associations, it is essential to understand the distribution of genetic variation among species that differ in these traits.

**Table 7.1 Characteristics of asterinid life histories including larval environment, presence of external feeding, life history four state character code, egg diameter, arm radius and lastly the dummy variable associated with the four state life history mode. Each dummy variable represents one transition in the four state life history mode. Each dummy variable represents an independent two state character.**

| Species | Larval Environment | Feeding | Life History | Egg Diameter (um) | Arm Radius (cm) | Dummy variable |
|---|---|---|---|---|---|---|
| *M. mortensoni* | Planktonic | feeding | 1 | 240 | 37 | 1 1 1 |
| *P. vivipara* | Benthic | non-feeding* | 4 | 150 | 15 | 0 0 0 |
| *P. parvivipara* | Benthic | non-feeding* | 4 | 235 | 3 | 0 0 0 |
| *P. pectinifera* | Planktonic | feeding | 1 | 170 | 40 | 1 1 1 |
| *P. exigua* | Benthic | non-feeding | 3 | 390 | 10 | 0 1 0 |
| *M. calcar* | Planktonic | non-feeding | 2 | 413 | 45 | 1 1 0 |
| *P. miniata* | Planktonic | feeding | 1 | 169 | 85 | 1 1 1 |
| *C. hystera* | Benthic | non-feeding | 4 | 440 | 12 | 0 0 0 |
| *C. pentagona* | Planktonic | non-feeding | 2 | 413 | 17 | 1 1 0 |

**Table 7.2 Population genetic parameters of each species used in the comparative analysis. # Populations is equal to the number of populations sampled for a species.**

| Species | # of Populations | Fst | Heterozygosity | Alleic Richness |
|---|---|---|---|---|
| *M. mortensoni* | 3 | 0.07 | 0.4 | 5.33 |
| *P. vivipara* | 3 | 0.12 | 0.002 | 1.189 |
| *P. parvivipara* | 4 | 0.73 | 0.002 | 1.31 |
| *P. pectinifera* | 3 | 0.04 | 0.37 | 8.13 |
| *P. exigua* | 11 | 0.76 | 0.08 | 2.98 |
| *M. calcar* | 13 | 0.33 | 0.39 | 6.51 |
| *P. miniata* | 12 | 0.12 | 0.43 | 5.15 |
| *C. hystera* | 6 | 1 | 0 | 1.4 |
| *C. pentagona* | 6 | 0.2 | 0.2 | 3.8 |

**Table 7.3 Results GLS tests of the correlation between life history characters (LH, AR, ED) and population genetic variation (Fst, Rch, Het). D1, D2, D3 represent the dummy variables, all three variables were covariates in each separate analysis. The notation is as follows y~x, where x (life history trait) is the predictor and y (genetic parameter) is the response variable. Three models were tested the GLS with lambda (λ) fixed at zero (λ = 0) and GLS lambda inferred from the model (λ = ML) Models with significant slopes are in bold text. Models with a superior AICc value are highlighted in grey. A test for model fit was conducted using AICc values**

| Character | λ=0 | | | | λ=ML | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Slope | SE | logL | AICc | Slope | SE | lambda | logL | AICc |
| Fst~LH | 3.27 | 1.96 | -26.36 | 59.29 | **5.08** | **2.13** | **0.49** | **-25.89** | **61.78** |
| Rch~LH | **-0.52** | **0.06** | **-3.01** | **12.59** | **-0.51** | **0.09** | **0.50** | **-3.28** | **14.56** |
| Het~LH | -5.29 | 2.66 | -28.50 | 63.57 | **-7.59** | **2.92** | **0.44** | **-28.15** | **66.30** |
| Fst~AR | -2.61 | 2.91 | -26.87 | 60.31 | -2.82 | 3.06 | 0.11 | -26.83 | 63.67 |
| Rch~AR | **0.57** | **0.17** | **-7.04** | **20.66** | **0.67** | **0.08** | **-0.63** | **-6.38** | **22.77** |
| Het~AR | 4.08 | 4.14 | -29.32 | 65.21 | 4.11 | 4.15 | 0.01 | -29.32 | 68.65 |
| Fst~ED | 9.72 | 6.00 | -25.30 | 57.18 | **13.20** | **5.37** | **-0.69** | **-24.69** | **59.38** |
| Rch~ED | -0.04 | 0.63 | -9.58 | 25.72 | 0.11 | 0.60 | 0.63 | -8.51 | 27.03 |
| Het~ED | -9.76 | 9.39 | -28.45 | 63.49 | -14.19 | 7.29 | -0.63 | -27.69 | 65.38 |
| Fst~D1 | -1.71 | 10.59 | | | -19.47 | 10.71 | | | |
| D2 | -6.19 | 9.98 | -19.32 | 49.22 | -2.18 | 7.91 | 1.40 | -16.43 | 46.86 |
| D3 | -2.02 | 7.89 | | | 0.21 | 8.34 | | | |
| Rch~D1 | 0.51 | 0.29 | | | **0.67** | **0.25** | | | |
| D2 | **0.82** | **0.28** | -1.30 | 12.85 | **0.84** | **0.26** | -2.59 | -0.46 | 14.92 |
| D3 | 0.20 | 0.22 | | | 0.12 | 0.16 | | | |
| Het~D1 | 1.52 | 13.66 | | | 24.68 | 11.33 | | | |
| D2 | 13.94 | 12.88 | -20.59 | 51.76 | 8.69 | 8.36 | 1.41 | -14.93 | 43.86 |
| D3 | 0.51 | 10.18 | | | -2.56 | 8.83 | | | |
| Fst~FN | -6.53 | 5.31 | -25.92 | 58.42 | -10.75 | 5.17 | 0.51 | -25.51 | 61.02 |
| Rch~FN | **0.94** | **0.39** | **-7.68** | **21.93** | **0.91** | **0.20** | **0.95** | **-6.88** | **23.76** |
| Het~FN | 9.75 | 7.57 | -28.39 | 63.36 | 13.44 | 7.69 | 0.35 | -28.22 | 66.43 |
| Fst~PB | -7.56 | 5.12 | -25.65 | 57.89 | **-13.28** | **5.05** | **0.50** | **-24.69** | **57.39** |
| Rch~PB | **1.25** | **0.23** | **-4.05** | **14.67** | **1.24** | **0.24** | **0.14** | **-4.04** | **18.08** |
| Het~PB | 12.29 | 7.02 | -27.87 | 62.31 | **31.65** | **5.97** | **1.43** | **-12.02** | **34.04** |

S

**Figure 7.1 Pruned and rate smoothed asterinid phylogeny used in the comparative analysis in APE. All species but one(*Patiria miniata*) were examined in the population genetic analyses in Chapter 4.**

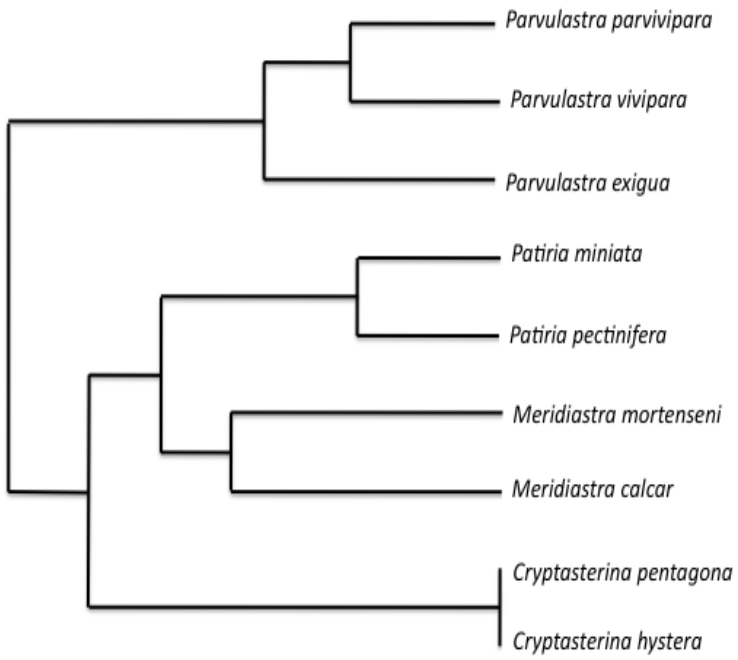
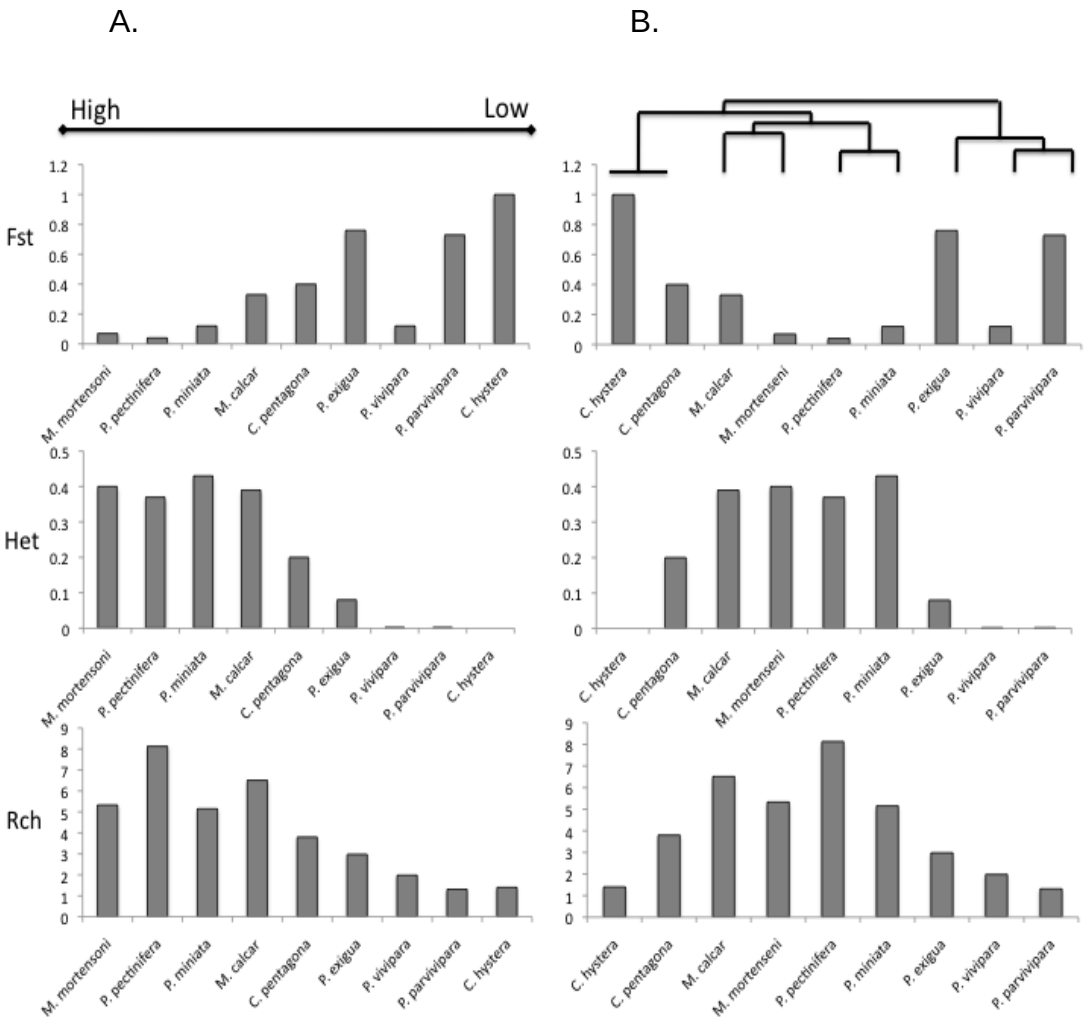


**Figure 7.2 Graphical representation on the relationship between dispersal mode and population genetic structure and the relationship between phylogeny and population genetic variation. Values on the y-axes are measures of population genetic variation. Values on the x-axis are species aligned by dispersal potential and phylogenetic distance.**



# Chapter 8 **SYNTHESIS**

## .Summary of Results

In this thesis I find that intrinsic life history characteristics, especially the mode of larval development and the mode of fertilization, can be fundamental in driving patterns of population genetic structure of the Asterinidae, but that constraints associated with phylogenetic relatedness and extrinsic factors such as historical vicariance and contemporary ocean currents are also important drivers of genetic variation.

In chapter 2 I examined the population genetic structure of *Patiria miniata.* This species is broadly distributed throughout the intertidal and subtidal northeast Pacific. Climatic fluctuations throughout the Pleistocene have driven patterns of population genetic variation among many intertidal community members in this region (Hewitt 2004, Marko et al. 2010). However, intrinsic dispersal mode can either act in concert with or override historical effects (Arndt and Smith 1998; Harley et al 2005). I find that despite the large geographic disjunction in the range of *P. miniata*, there is a low level of population genetic structure across this 900-kilometre expanse, which suggests high levels of gene flow via planktonic larvae in some parts of this species range. However, I find extrinsic forces associated with historical vicariance cause a high level of population genetic differentiation across queen Charlotte Sound. This chapter gives preliminary evidence that a species' intrinsic dispersal ability as well as its response to extrinsic factors can drive patterns of genetic structure.

In Chapter 3 I was able to design microsatellite markers that amplified consistently, and with minimal abiotic bias, in all 9 asterinid species in my study. I used these markers in Chapter 4 to describe population genetic parameters of each species in my analysis. I find that population genetic parameters such as heterozygosity, $F_{is}$, and Allelic Richness appear to be highly associated with dispersal mode and mating system, whereas patterns of between population genetic variation such as $F_{st}$ are slightly less consistent in their relationship with intrinsic life history characteristics and in some cases can be reliably attributed to extrinsic factors.

In Chapter 5 I examined the mating systems and describe characteristics of brooding of two live bearing asterinid species used in my population genetic analysis (*P. parvivipara,* and *P. vivipara*). One goal of this chapter was to determine the relative role of outcrossing and self-fertilization in levels of within-population genetic variation. I was also interested in the characters that could best predict clutch size and offspring size and their associations with sibling cannibalism and parental size constraints in the two species. I find there is at least some sexual reproduction in *P. parvivipara,* and that in two broods at least one offspring was the result of sexual reproduction by outcrossing. However a lack of variation in broods of *P. vivipara* precluded me from drawing any conclusions about paternity or sexual vs. asexual reproduction in this species. Despite my inability to conclusively determine the extent of self-fertilization vs. outcrossing in these populations, I found a striking relationship between clutch size and offspring size in *P. parvivipara* broods that may ultimately be related to

sibling cannibalism. In this species larger parents, on average, produce larger clutches, however the decline in clutch size with increasing offspring body size is more severe in larger parents (that generate larger clutches), suggesting that larger parents have a greater decrease in fitness from the combine effects of brooding and sibling brood cannibalism than small parents. This pattern addresses, and in some respects answers, a two-decade-old question of the prevalence of brooding in small sized marine invertebrate species (Strathmann and Strathmann 1982).

In Chapter 6 I tested the utility of Bayesian ancestral character state reconstruction in inferring patterns of evolution of life history throughout the Asterinidae. I also used my analysis to evaluate the utility of ancestral character reconstruction in comparative analyses. I found that without a strong model of life history evolution from first principles, it will be difficult to infer reliable ancestral character states because reconstructions are highly variable depending on the model of character evolution I use. Ultimately I learned that using ancestral character states in comparative analyses would require many assumptions about the model of character evolution, which are known to drastically change the results of a reconstruction.

Finally, In Chapter 7 I used a comparative analysis that accounts for phylogenetic relatedness among species in the quantitative analysis of the relationship between life history and population genetic structure. I used a generalized least squares model and assess model fit using AIC values. I examined model fit using different levels of phylogenetic signal in the correlation

among characters, including a Brownian motion model, where characters are completely constrained by phylogeny, a model with an intermediate maximum likelihood value of phylogenetic signal among characters and a model in which phylogenetic relatedness is not accounted for in the comparative analysis. I found that in many cases life history was significantly related to population genetic structure but that the degree to which there is a better model fit when accounting for phylogenetic signal varies among characters.

## .Retrospective Thoughts and Future Directions

In this thesis I was able to determine the relative influence of life history characteristics on population genetic structure in the Asterinidae. However, I was only able to infer differences in patterns of frequency-based genetic variation among species, which prevented me from drawing any conclusions about differences in actual levels of gene flow among species with different dispersal potentials. Further sampling of nuclear and mitochondrial sequence data would allow me to determine how levels of gene flow (measured using coalescent programs such as IM and Migrate) (Beerlie and Felsenstein 1999, Hey and Nielsen 2004) differ between species with different mating systems and dispersal potentials.

Similarly, the lack of uniform stepwise mutation model among my microsatellite markers made them unfit for coalescent-based analyses that examine divergence times among populations and among species (Selkoe and Toonen 2006, Hellberg 2009). Nuclear and mitochondrial sequence data would

allow me to estimate divergence times and population sizes using a coalescent based approach. Ultimately, these data would help me to accurately match divergence times and patterns of migration and population size to the timing and magnitude of extrinsic forces such as glacial cycles and ocean currents to conclusively determine their relative influence on population genetic structure.

I have been fortunate to have a group of collaborators with whom I worked on this project. My collaborators from the University of Hawaii and the University of California at Davis are examining patterns of polymorphism among nuclear and mitochondrial sequence markers, which can be used for coalescent based tests of migration, mutation, and divergence times.

One distinct weakness of the comparative analysis is that there are only nine data points with which to infer a relationship between life history and population genetic structure. Surprisingly, despite the small sample size I found strong correlations between some life history variables and population genetic parameters. Other results were more ambiguous and may have benefited from a larger sample of species. Unless a clear pattern exists, the scarcity of data points can greatly decrease one's power to effectively estimate a regression line. In some instances, phylogeny accounted for some proportion of the residual error in my data, improving model fit, while in other cases it had little effect on the relationship or worsened it.

Analyses such as mine are rarely conducted on more than 10 closely related species because the data are excessively time consuming to collect and require extensive analyses. Instead, many authors (Bohonak 1999, Wiersing and

Toonen 2008) examine these relationships using a review-style or meta-analytical approach that uses data from many smaller studies to draw conclusions about the effects of life history on population genetic structure. While these studies are extremely useful they are weakened by the fact that many different marker types and sampling designs are used in each independent analysis. For this reason conclusions from some meta-analyses may be influenced by marker type. For example differing levels of polymorphism among markers types can lead to generally different estimates of within- and between-population genetic variation. Measures of population genetic variation using microsatellite markers will have overall higher levels of allelic diversity (due to the high mutation rate of these markers) (Balloux and Moulin 2002), but lower absolute levels of population genetic structure, measured as *F*st, due to this high variation (Selkoe and Toonen 2006). Ultimately, a study such as mine would benefit from a greater sample of species and marker types. With extended sampling a clearer picture of the influence of life history on patterns of population genetic variation may emerge.

## .Concluding Remarks

This work contributes to a large body of research on comparative population genetics and phylogeography examining the relative role of life history in the population genetic structure of marine organisms (Duffy 1993, Hellberg 1996, Edmands and Pots 1997, Arndt and Smith 1998, Bird et al. 2007, Lee and Boulding 2009). Although these ideas have been tested in many groups of

marine and aquatic organisms, we would benefit from knowing more about how the parallel evolution of life history forms or shared ancestral similarities in intrinsic dispersal model among groups of closely related organisms. This information would enable phylogeographers to compare data points among a diversity of life history types that share similar evolutionary histories and ecological attributes that could otherwise confound analyses. With additional analyses on a diversity of species groups, similar to that in the Asterinidae, we may be able to gain a clearer picture of the influence of intrinsic life history characters on higher-order processes such as local adaptation, speciation, and extinction that are associated with patterns of population genetic variation in marine systems (Grobserg and Cunningham 2001).

## .Appendices

EDT6385_CarsonKeever_appendix1
EDT6385_CarsonKeever_appendix2
EDT6385_CarsonKeever_Appendix 3
EDT6385_CarsonKeever_Appendix 4
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