A SEARCH FOR INTROGRESSION AND GENE FLOW AMONG POPULATIONS OF THREE PACIFIC COAST *ANTHOPLEURA* SPECIES

by

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Abstract

Three species of anemones, *Anthopleura elegantissima, Anthopleura sola* and *Anthopleura xanthogrammica* were studied at nine sites along the Pacific Coast of the United States to determine the extent of genetic isolation among the different species and among populations of the same species. At each site, a detailed morphological analysis was taken from a representative group of each of the species present. Tissue samples for DNA analysis were also taken from a subgroup of these. Morphological distinctiveness was assessed by discriminant function analysis. Phylogenetic relationships were examined based on a sequence analysis of a portion of the Arginine Kinase gene which included exon and intron sections. Locus-by-locus comparisons were made among variable loci to determine the extent of differences among the species. Gene flow among populations was assessed by AMOVA and F_{ST}. Both morphological and genetic analysis indicated that *A. xanthogrammica* is easily distinguishable from both *A. elegantissima* and *A. sola*, while the latter two were far more difficult to separate. Although *A. sola* seems to morphologically combine characteristics of the other two species, it had a number of unique genetic polymorphisms not seen in either of the other species. F_{ST} indicated that there is a high degree of gene flow among all populations but strong barriers to gene flow among all the species. This indicates that the three species are freely interbreeding within their respective populations along the coast but are distinct species which likely diverged from a common ancestor recently.

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INTRODUCTION

Marine species along the Northeast Pacific coast are distributed in such a way that the region can be divided into three biogeographic provinces: the Aleutian Province, the Oregon Province, and the California (or San Diego) province. Depending on the authority, the Aleutian Province begins anywhere from Oregon to the Alaska Peninsula and spreads north to the Bering Strait. The Oregon Province stretches from the Aleutian Province south to Point Conception, CA. The northern boundary of the Oregon province has been disputed for some time, with some arguing that the true boundary lies between northern Washington and southern British Columbia (Hartman and Zahary, 1983). The southern boundary is generally accepted as Point Conception (Hartman and Zahary, 1983), though some species ranges extend north or south past it. The California Province, which extends from Point Conception, CA south to the Pacific coast of Baja California, is a unique area where both northern and southern species mix (Briggs, 1974). It consists of warm-temperate waters which allow for a mix of both warm- and cold-derived species.

The segregation of species into these provinces can be better understood in context of the currents along the Pacific coast of the United States. The dominant current in this region is the California current (Figure 1), which is part of the North Pacific Gyre. As this gyre approaches the West coast of North America, it is referred to as the North Pacific Current (Pickard and Emery, 1982). This current comes ashore near the coast of Northern Washington and there splits into two parts. One part turns north and forms the Alaskan Gyre in the Gulf of Alaska. The other part turns south and forms the California

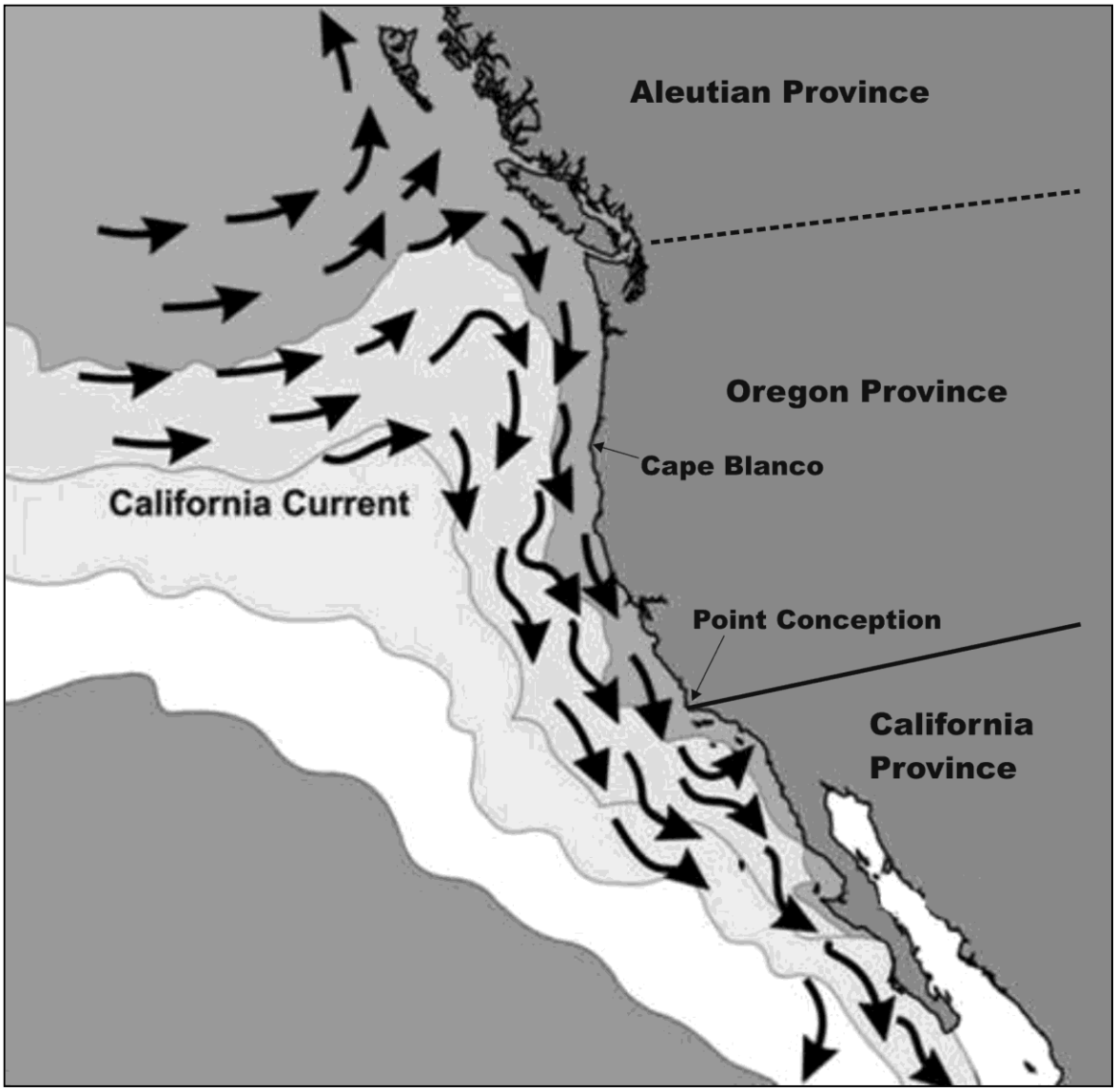

Figure 1. Average flow of the California Current along the Pacific Coast of North America (adapted from SFSC.noaa.gov).

Current which stretches from the continental shelf to far offshore. This southern-flowing portion of the current is where the Oregon biogeographic province is found.

Physical features of the coastline in this region also have an impact on water flow. The northern area skirted by the California Current has a fairly uniform coastline which allows the current system to flow mostly uninterrupted. However, various capes along the central region (including central Oregon, and northern and central California) can interrupt water transport. The geometry of Cape Blanco in central Oregon results in differential water flow north and south of this major cape (Pierce *et. al.*, 2000; US GLOBEC, 2002). North of Cape Blanco nutrient rich water remains close to shore, but as the water moves south, it eventually interacts with Heceta Bank and a jet of water is diverted offshore. South of Cape Blanco, another jet of water is displaced up to several hundreds of kilometers offshore (Pierce *et. al.*, 2000; US GLOBEC, 2002). Cape Mendocino in northern California contributes to stronger upwelling in central California through Point Conception (Connolly and Roughgarden, 1998; Pierce *et. al.*, 2000; US GLOBEC, 1994). At Point Conception, the California Current veers offshore where it eventually joins the North Equatorial Current (Pickard and Emery, 1982). The biogeographic region south of this point is the California province.

Coastal winds and the southward flow of the California Current often produce upwelling. Upwelling occurs when surface waters move offshore, triggering a rise of cold, nutrient-rich water to the surface near shore. Upwelling is variable along the coast due to wind and the physical structure of the coastline. North of Cape Blanco, strong winds contribute to moderate upwelling in the spring and summer and strong downwelling in the winter (Strub and James, 2000; U.S. GLOBEC, 1994). The region

between Cape Blanco and Point Conception has the most seasonal variation in winds with strong upwelling-favorable wind in the spring and summer, while in the winter, storms drive the current poleward with downwelling-favorable winds. South of Point Conception, where winds are minimal, upwelling is favored all year-round (Strub and James, 2000; U.S. GLOBEC, 1994).

Aside from upwelling events, the California Current shows large-scale seasonality as well. From late spring through early fall, water flows strongly to the south; however, in the winter net flow can be somewhat weak and variable (Marchesiello et al., 2003) (Figure 2). Variation also exists in the form of poleward flowing undercurrents. The California Undercurrent is a poleward flowing, subsurface (250m deep) current which is present most of the year. It does not exceed distances of approximately 100 km from the coast (Lynn and Simpson, 1987; Pierce et al., 2000). The Inshore Countercurrent, also known as the Davidson Current, consists of poleward flowing water near the coast in the winter north of about 33˚N (Strub and James, 2000). El Niño events tend to result in a stronger poleward component of the California Current system (Hayward, 1993).

This variability in current and upwelling along our coast could have important implications for gene flow among populations. Most intertidal species, including anemones, have a pelagic larval stage. Pelagic larvae are important for promoting genetic exchange across broad geographic areas and facilitate expansion of species' ranges. In general, larvae are too small to swim against a major current and so are carried along in the direction the current is moving. The direction of current flow thus has a strong impact on dispersal patterns of these organisms. The south-flowing California

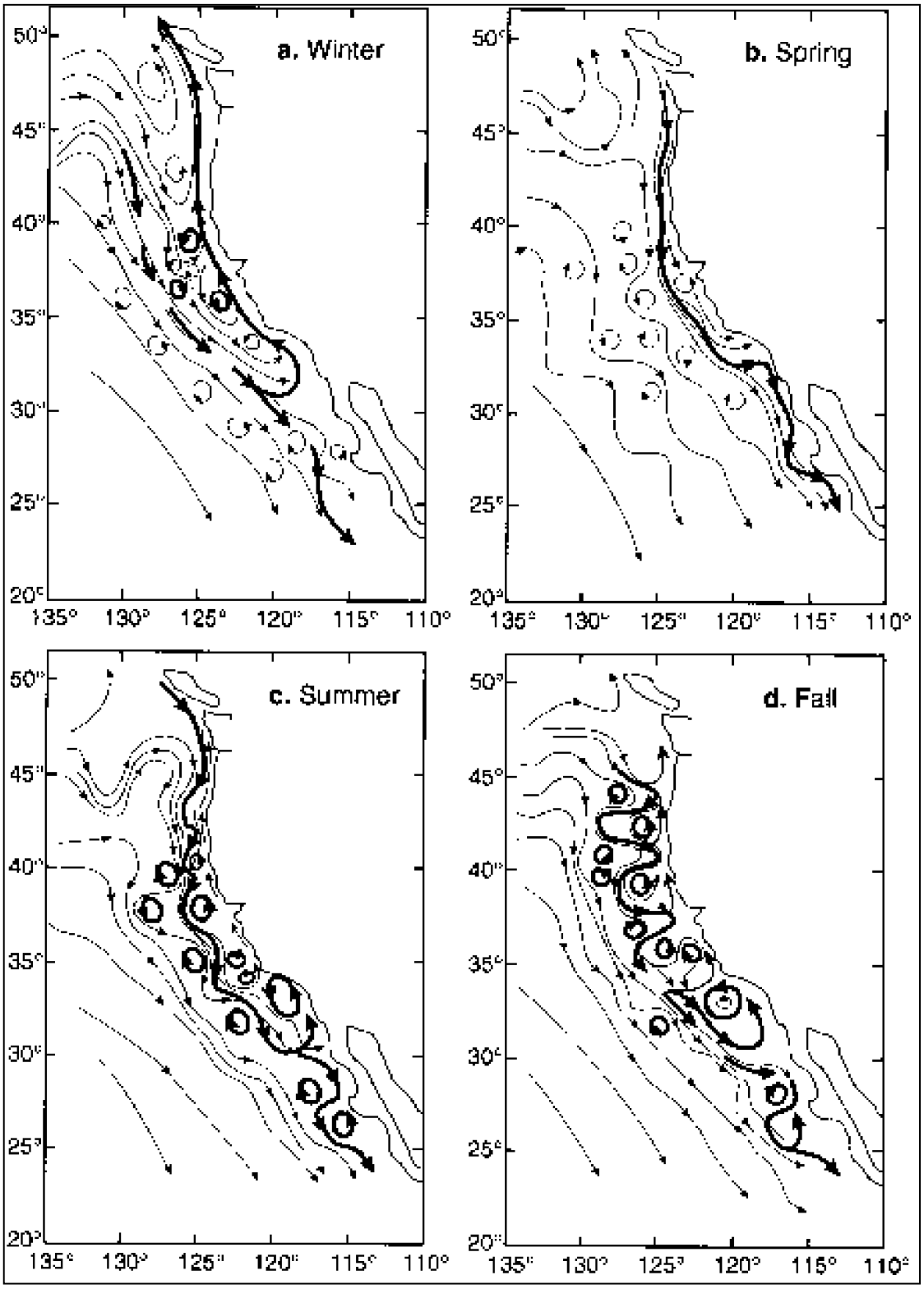

Figure 2. Seasonal variations in circulation of the California Current (taken from Strub and James, 2000).

Current along the Washington, Oregon and California coast makes it easy for larvae to disperse to the south. On a local scale, standing eddies located near many of the major capes as well as local headlands (Morgan *et. al.*, 2011; Marchesiello et al., 2003) can favor recruitment of larvae into these areas. The California Undercurrent may allow northward larval dispersal at times. However this current is strongest in the winter, when few larvae are dispersing. After El Niño events and at certain locations where the Davidson Current is strong, northward transport of larvae may occur. The net result of these current systems is increased potential for species distribution all along the coast in the Oregon province. Point Conception, where the California Current veers offshore, tends to be the southern limit for many species in the Oregon province (Hartman and Zahary, 1983).

As previously mentioned, areas in the central region of the Oregon province tend to have stronger upwelling and jets which disperse surface water offshore due to the effect of wind in the area as well as the geometry of the various capes (US GLOBEC, 1994; US GLOBEC, 2002; Connolly and Roughgarden, 1998). These regions tend to have lower intertidal recruitment (Connolly et al., 2001), which could be explained by larvae being swept offshore by these jets and upwelling instead of making it into the intertidal zone to settle. However, these features (jets and offshore surface transport) have only been studied in terms of their effects on local recruitment. The effects on wide-scale larval dispersal and gene flow along the coast have not yet been investigated, but they could potentially be interrupting this type of dispersal and gene flow as well. Because larvae are being transported offshore in these areas, areas of upwelling and jets could provide a partial barrier to gene exchange within the Oregon province.

Anemones of the genus *Anthopleura* are important intertidal species which can be found spanning all three Pacific Coast biogeographic provinces. Within this genus, three species are especially prominent: *Anthopleura elegantissima*, *Anthopleura sola*, and *Anthopleura xanthogrammica* (Figure 3, Table 1).

Anthopleura elegantissima (Brandt, 1835) (Figure 3a) is a small (3-3.5 cm maximum diameter) anemone with a range extending from Alaska to Baja California (Hand, 1955; McFadden *et.al.* 1997). Besides reproducing sexually, a process which involves a pelagic larva, it also reproduces asexually by longitudinal fission. This mode of asexual reproduction does not involve any larval stage. The species can be found in asexually-produced aggregations numbering up to 100,000, though most aggregations are much smaller (Hand, 1955; Pearse and Francis, 2000). *A. elegantissima* typically has a green column and pink or lavender tipped tentacles and obvious mesenterial insertions visible as a color pattern on the oral disc (Hand, 1955) (Table 1). Its column is covered with simple verrucae (verrucae are adhesive tubercles; compound verrucae are forked and have several adhesive patches) along much of the column, but they become compound near the collar of the anemone. Its verrucae are usually in vertical rows. It is host to symbiotic zoochlorellae in the northern part of its range and to zooxanthellae throughout its range (Pearse and Francis, 2000). *A. elegantissima* is commonly found on exposed rocks and ledges fairly high in the intertidal zone and tends to prefer areas with high wave action (Hand, 1955). Around San Francisco *A. elegantissima* releases gametes into the water in late spring through early fall, and in Washington, in September and October (Morris et al., 1980).

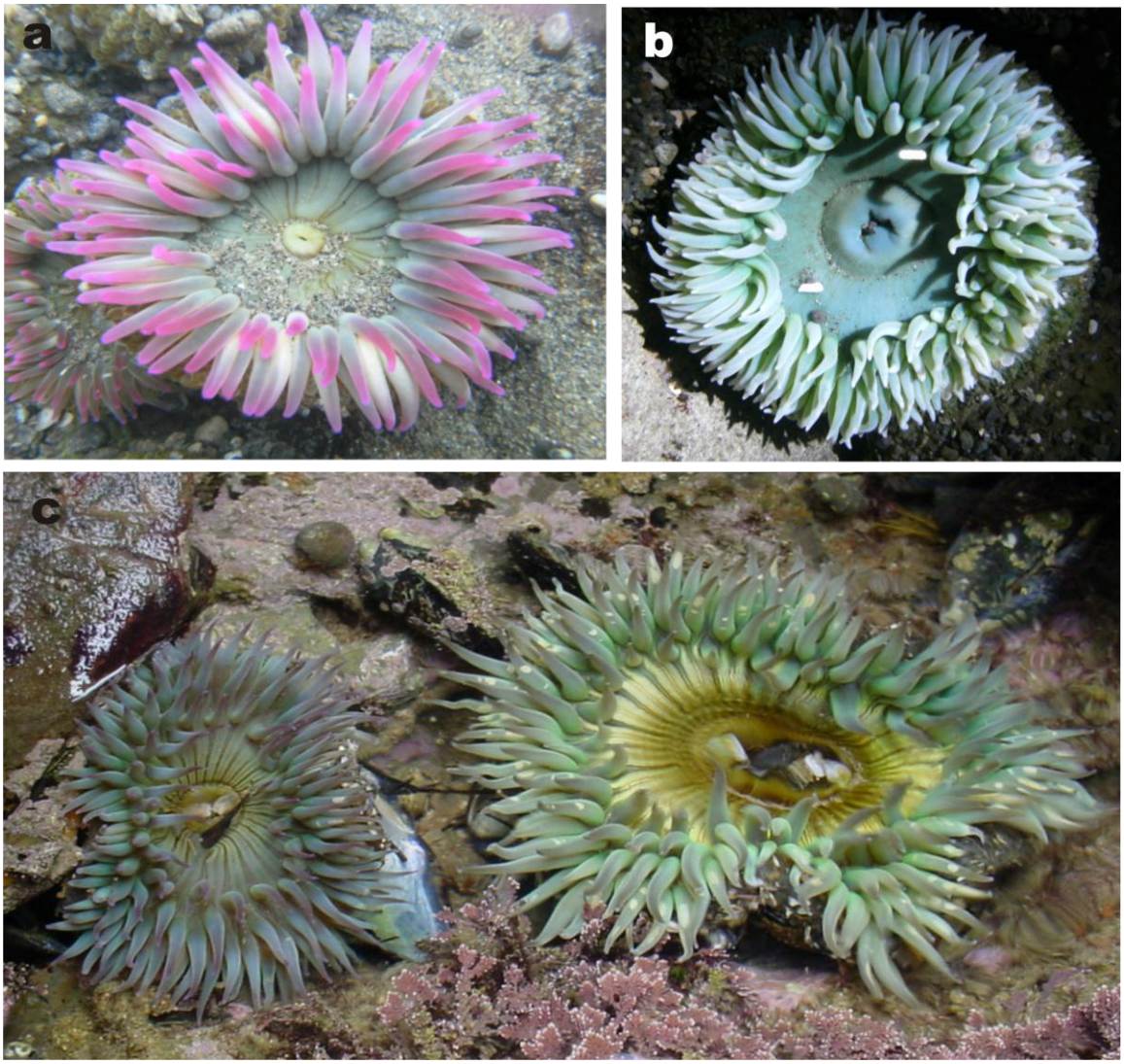

Figure 3. (a) *Anthopleura elegantissima*, (b) *Anthopleura xanthogrammica*, and (c) *Anthopleura sola* (*A. sola* photo by David Cowles).

Table 1. Distinguishing characteristics of the three Pacific Coast *Anthopleura* species in this study.

	A. elegantissima	***A. sola***	***A. xanthogrammica***
Maximum Diameter (cm)	3.5	25	20
Mode of Reproduction	Clonal, Sexual	Sexual	Sexual
Sociality	Large clonal aggregations of tightly packed individuals	Solitary, may touch but not tightly packed	Solitary, may touch but not tightly packed
Typical Habitat	High intertidal, exposed rocks	Mid to low intertidal	Low intertidal, in crevices and tidepools below mussels protected from dessication
Oral Disk:			
Pattern	Stripes at insertion of mesenteries	Stripes at insertion of mesenteries	Plain green - few stripes or faint
Lips	Often loose and frilly	Often loose and frilly	Usually held tight
Tentacles:			
Color of Base	Green	Green	Green
Color of Tip	Pink or Lavender	Pink or Lavender	Gray-Green or Blue
White Spots	Sometimes Present	Sometimes Present	Usually Absent
Column:			
Verrucae Pattern	Vertical Rows	Vertical Rows	Scattered
Verrucae Type	Mostly simple, some compound near top of column	Mostly simple, some compound near top of column	Many compound
Foot	Expands well beyond column	Expands well beyond column	Similar diamter as column

Anthopleura sola (Pearse and Francis, 2000) (Figure 3c) is found from Baja California to just north of San Francisco Bay, California (Francis, 1979; McFadden *et.al.* 1997). It is on average much larger than *A. elegantissima*, and can grow to 20-25 cm in diameter. Aside from size, *A. sola* is morphologically very similar to *A. elegantissima* and in fact up until it was designated a separate species in 2000 it was simply considered to be a large solitary form of *A. elegantissima*. However, in the field it can generally be distinguished by its larger size, lack of fission scars, solitary lifestyle, its habitat, and its intertidal and geographic range (Pearse and Francis, 2000). *A. sola* is more commonly found in relatively sheltered positions, often attached to rocks buried in the sediment so that its column is partly or completely hidden (Hand, 1955; Pearse and Francis, 2000). Zooxanthellae are typically found as endosymbionts in *A. sola*, while zoochlorellae have never been documented in this species (Pearse and Francis, 2000). Since this species has only been recently identified, it is not specifically known when their gametes are released, but it is likely safe to assume that they spawn mostly during the summer as do the other two species.

Anthopleura xanthogrammica (Brandt, 1835) (Figure 3b) is found from Japan through Alaska and down to southern California (Hand, 1955), but south of Point Conception it is restricted to only a few areas where water temperature is cooler (Francis, 1979). It can grow to about 20 cm in diameter with its extended column reaching up to 25 cm in height (Hand, 1955); however it is usually closer to 10-13 cm across its oral disc. Adults seem to mainly inhabit tidepools and fissures in the lower intertidal, just below the mussel zone. They show preference for areas of strong wave action (Hand,

1955). *A. xanthogrammica* is uniformly green, even on its tentacles, and rarely shows a color pattern due to mesenterial insertions on its oral disc (Hand, 1955; Pearse and Francis, 2000) (Table 1). Unlike *A. sola* and *A. elegantissima*, the column of *A. xanthogrammica* is covered in compound verrucae which are not usually distributed in longitudinal rows (Hand, 1955; Pearse and Francis, 2000). Some young specimens of *A. xanthogrammica*, however, may have verrucae arranged in vertical rows on at least part of the column. Despite their distinct morphological differences, *A. sola* is commonly confused with *A. xanthogrammica* in the field; in fact they are so often misidentified that "caution is indicated in interpreting older literature" (Pearse and Francis, 2000). *A. xanthogrammica* releases gametes into the water in late spring through summer (Morris et al., 1980).

A. xanthogrammica is characteristic of the northern portion of the Oregon province, *A. sola* of the southern portion, and *A. elegantissima* is easily found throughout. However, several observations make this distribution pattern less clear. In Washington, *A. xanthogrammica* is clearly distinguishable from other species in the area. Likewise, *A. sola* is easily distinguished in southern California. However, north of San Francisco, at the central portion of the Oregon province, these species can sometimes be more difficult to distinguish. Some *A. xanthogrammica* and *A. sola* seem to have intermediate features, which some reports (Francis, 1979) suggest could be due to hybridization. Others have noted that even *A. elegantissima* can sometimes be difficult to distinguish in this area (Cowles personal observations), due, for example, to unusually large size.

Several models of speciation may explain these observations. First, the three species may be fully distinct everywhere. According to this model, there is minor

variation present within each species that has led to anecdotal confusion in the northern California region. A second possible model may be that introgression is occurring between two or more of the species in the central to northern California area. Finally, a third model is that the northern California area is a center of radiation for these species. *A. xanthogrammica*, a northern branch, and *A. sola*, a southern branch, may have their origin in northern California and be hard to distinguish there. According to this model, the apparent species differences seen clearly to the north and south may be due to clinal variation along these trajectories. Both species may also be interbreeding with *A. elegantissima* in this area. All of these possibilities may be affected by limited gene exchange to the north caused by the net southward flow of the California Current and the recruitment barriers posed by upwelling. Distinct genetic divisions can occur within widely distributed species, especially those that span biogeographic provinces (Hedgecock, 1994). Both *A. elegantissima* and *A. xanthogrammica* are widely distributed organisms which could be influenced by this phenomenon. In this paper I propose to test the hypothesis that *A. xanthogrammica, A. sola* and *A. elegantissima* are indistinguishable across their range both genetically and morphologically. I will also determine if there are any consistent definable differences between populations of each species by comparing their morphology and genetics at several points along their range.

MATERIALS AND METHODS

Field Sites:

For this study I collected data from *Anthopleura* anemones at 9 sites ranging from Dana Point, CA to Anacortes, WA (Table 2, Figure 4). At each site I examined 10 to 15 individuals of each species, for a total of 144 individuals (Table 3). Thirteen morphological features were recorded for each individual (Table 4). These features were chosen based on personal observations of variable features and on descriptions of the species' distinguishing characteristics from the literature. Additionally, tissue samples were taken from the foot of at least 5 individuals of each species from each site for DNA analysis. Tissue samples were coded according to the pattern in Figure 5. The first anemone for analysis at each study site was chosen with the only qualifications being that it was able to be studied (tentacles, column, and oral disc visible), and each successive animal was chosen by walking at least 1.3m in any direction in order to minimize collection bias and to sample over a larger area. The first organism beyond 1.3m in that direction which was appropriate for analysis (tentacles, column, and oral disc were visible) was used. *A. elegantissima* samples were chosen using this method, with the additional requirement that they must appear to be from a separate clone. Distinctions between clones were made based on systematic differences in anemone color or size, or a distinct gap separating the individuals. At each site, I also looked for any individuals which did not fit the typical descriptions of the species and sampled those individuals as well.

Before any measurements were taken, each individual was photographed and the photo numbers recorded. Size measurements were determined to the nearest half

Table 2. Location and species present at each study site.

Site	Abbreviation	Latitude	Longitude	Species Present
Rosario Beach	RB (1)	48˚ 25.38	122˚ 40.35	*A. elegantissima*
Kalaloch	KL (2)	47˚ 39.17	124˚ 23.52	*A. elegantissima, A. xanthogrammica*
Yaquina Head	YH (3)	44˚ 40.53	124˚ 04.67	*A. elegantissima, A. xanthogrammica*
Seal Rocks	SR (3)	44˚ 29.74	124˚ 05.11	*A. elegantissima, A. xanthogrammica*
Bodega Bay	BB (5)	38˚ 19.00	123˚ 04.27	*A. elegantissima, A. xanthogrammica, A. sola*
Moss Landing	ML (6)	36˚ 48.35	121˚ 47.35	*A. elegantissima, A. xanthogrammica, A. sola*
San Simeon	SS (7)	35˚ 39.14	121˚ 14.53	*A. elegantissima, A. sola*
Moss Beach	MB (8)	33˚ 31.49	117˚ 46.14	*A. elegantissima, A. sola*
Dana Point	DP (9)	33˚ 27.59	117˚ 42.89	*A. elegantissima, A. sola*

Table 3. Sample size for each analysis used in this study.

Analysis	**Number Used**			
	A. elegantissima	*A. sola*	*A. xanthogrammica*	Total
Discriminant Function	60	49	35	144
Maximum Likelihood	36	9	22	67
Maximum Parsimony	36	9	22	67
F_{ST}	31	12	21	64
Chi Square of Variable Loci	31	12	21	64

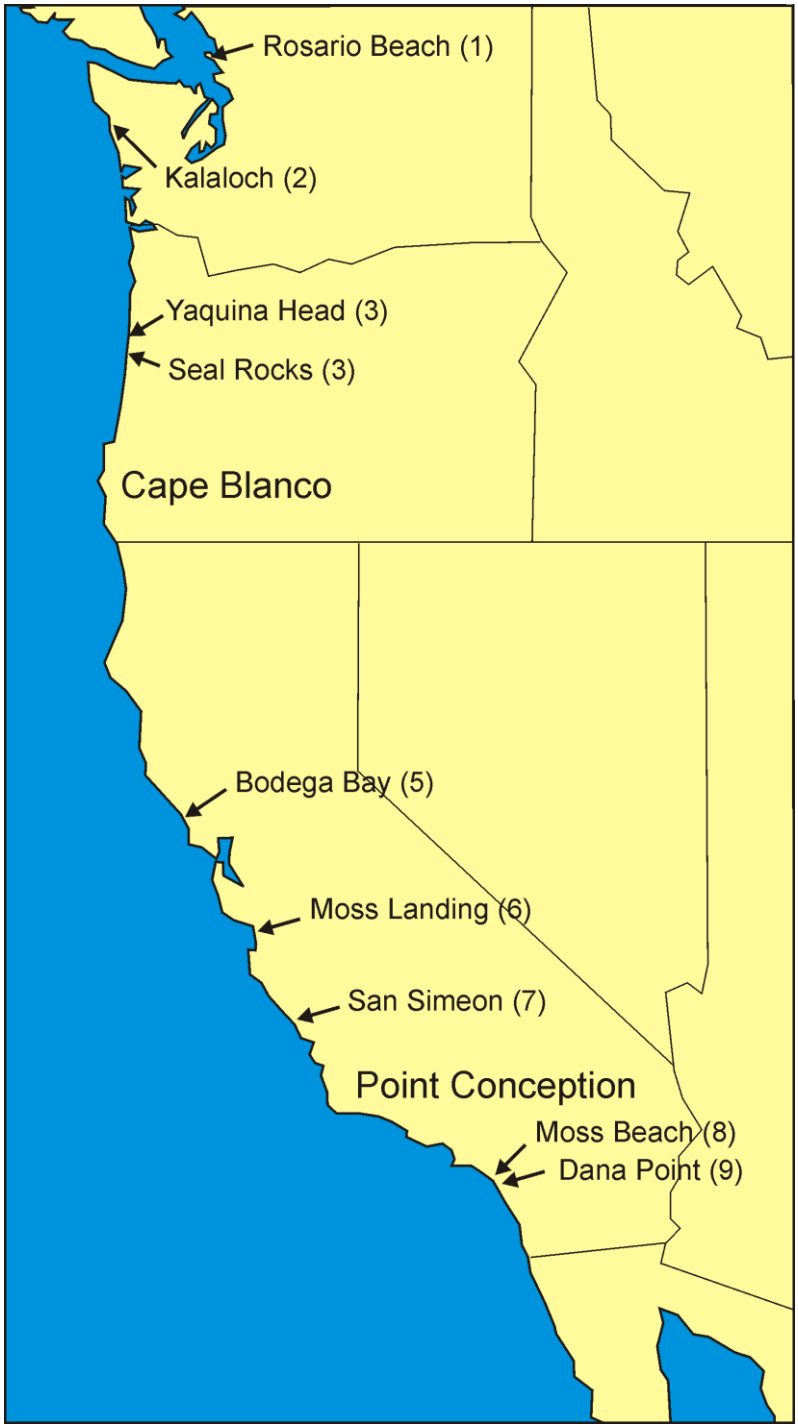

Figure 4. Map of study sites along the coast and potential geographic barriers to larval dispersal. See Table 2 for details.

Table 4. Summary of morphological measurements recorded for the three species of anemones. SPSS coding gives the numeric values used in SPSS for qualitative characteristics.

Feature	*Anthopleura elegantissima*				*Anthopleura sola*				*Anthopleura xanthogrammica*				SPSS Coding
	Mean	**Median**	**Minimum**	**Maximum**	**Mean**	**Median**	**Minimum**	**Maximum**	**Mean**	**Median**	**Minimum**	**Maximum**	
Oral Disc Diameter (mm)	26.9	24.0	12.0	63.0	87.0	88.0	36.0	134.0	83.8	83.0	37.0	125.0	
Oral Disc Pattern	1.7	2.0	0.0	3.0	2.2	2.0	2.0	3.0	0.7	1.0	0.0	2.0	0: None 1: Faint 2: Moderate 3: Strong
Tentacle length (mm)	7.4	6.0	3.0	24.0	19.0	20.0	8.0	35.0	13.2	12.0	4.0	27.0	
Tentacle Width (mm)	1.1	1.0	0.5	3.0	3.2	3.0	2.0	5.0	3.4	3.8	1.0	7.0	
Tentacle Length:Width	7.2	7.0	3.0	18.0	6.0	5.8	3.3	9.3	4.2	3.8	1.8	10.0	
Tentacle Tip Color	7.5	8.0	3.0	11.0	2.6	2.0	1.0	9.0	2.7	1.0	0.0	9.0	0: None 1: Grey 2: Grey/Brown 3: Brown or Tan 4: Blue 5: Blue/Green 6: Green 7: Purple/Grey 8: Pink 9: Purple 10: Pink/Purple 11: White
Tentacle Base Color	2.4	3.0	0.0	7.0	2.3	2.0	1.0	7.0	4.4	5.0	0.0	6.0	0: None 1: Grey 2: Grey/Green 3: Green 4: Lime 5: Blue/Green 6: Blue 7: Brown or Tan 8: Grey/Brown
Column Height	9.7	8.0	3.0	30.0	29.7	29.0	10.0	54.0	35.6	31.5	10.0	73.0	

Table 4 continued. Summary of morphological measurements recorded for the three species of anemones. SPSS coding gives the numeric values used in SPSS for qualitative characteristics.

Feature	*Anthopleura elegantissima*				*Anthopleura sola*				*Anthopleura xanthogrammica*				SPSS Coding
	Mean	**Median**	**Minimum**	**Maximum**	**Mean**	**Median**	**Minimum**	**Maximum**	**Mean**	**Median**	**Minimum**	**Maximum**	
Column Color	2.9	3.0	1.0	3.0	1.9	2.0	1.0	3.0	3.0	3.0	3.0	3.0	0: Clear 1: Pale green or Grey 2: Grey to Green/Grey 3: Green 4: Dark Green
Debris Cover (%)	25.8	20.0	0.0	90.0	40.6	40.0	0.0	95.0	26.5	17.5	0.0	90.0	
Verrucae Pattern	2.9	3.0	1.0	3.0	2.9	3.0	2.0	3.0	1.1	1.0	1.0	2.0	1: No pattern 2: Some Rows 3: Rows
Verucae Simple vs. Compound	1.8	1.0	1.0	3.0	1.4	1.0	1.0	3.0	2.9	3.0	1.0	3.0	1: Simple 2: Both Simple and Compound 3: Compound
Lips	2.5	3.0	1.0	3.0	1.3	1.0	1.0	3.0	2.4	3.0	1.0	3.0	1: Exposed (loose) 2: Partly Exposed 3: Pursed or Not Exposed
Foot Diameter (mm/side)	3.7	3.0	0.0	10.0	5.3	5.0	0.0	15.0	18.1	19.0	4.0	35.0	

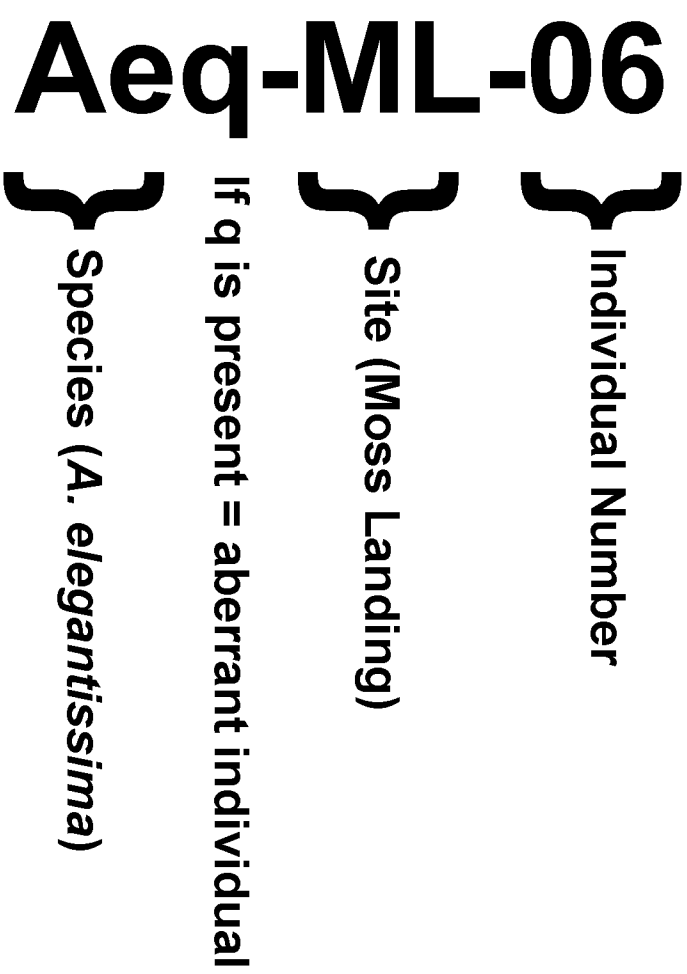

Figure 5. Example of coding for tissue samples.

millimeter using calipers. I attempted to keep color descriptions consistent between species, sites and individuals; however no standardized color chart was used. Oral disc pattern was separated into four categories: none, faint, moderate, and strong (Table 4). Oral disc width and foot spread were taken at the widest point of the anemone. In order to keep the values consistent, all column height measurements were determined after poking and harassing the anemone until it fully closed and contracted. Verrucae were considered to be in rows if most verrucae were aligned in groups of three or more arranged in vertical rows. Verrucae were considered compound if the tip of each verruca was divided into at least two segments with adhesive patches on each segment. Lips were considered 'loose' if all flaps were clearly visible outside the animal's mouth. If only part of the lips were visible they were considered 'partly loose'. If no lip flaps were visible but the mouth was gaping rather than tightly closed, it was classified as 'open mouth'. A closed mouth without the ridge and no exposed flaps was designated 'closed'. If the mouth was closed and the lips were not exposed but a distinct ridge was visible around the mouth, the anemone's lips were considered 'pursed'. Debris percent cover on the column was estimated to the nearest 5% for each individual before the anemone was forced to close. Finally, GPS coordinates were taken for each individual using a Garmin GPSmap 76® handheld GPS with WAAS (Wide Area Augmentation System) enabled for greater precision.

Tissue samples were collected using a razor blade or scalpel and immediately placed in 95% ethanol. Between samples of the same species at the same site, the razor blade/scalpel was cleaned by wiping with rubbing alcohol or 95% ethanol. Separate

razor blades or scalpels were used for the different species. Tissue samples were stored in 95% ethanol at room temperature until they could be analyzed in the lab.

Morphological Analysis:

Morphological Features:

Morphological features which were counts or direct measurements were recorded directly in an SPSS data sheet. Features which were categorical were numerically coded for use in the data sheet (Table 4).

Discriminant Function Analysis:

The morphology of the anemones was compared among the species and among the sites along the coast in a series of several steps. First, to identify which individuals could be unambiguously identified to species I chose calibration sites for each species. The calibration sites were sites in which the species either was found exclusively, or if no such sites existed I chose sites removed as far as possible from sites with individuals of another species which could potentially be confused with the species of interest and individuals which had well-defined and distinctive features. For *Anthopleura elegantissima* the calibration site was Rosario Beach, WA. For *Anthopleura xanthogrammica* the calibration site was Kalaloch, WA. For *Anthopleura sola* the calibration sites were Dana Point and Moss Beach, CA. For the first discriminant function analysis I identified the calibrated species only at these sites and left all other individuals unknown, allowing the discriminant function to assign species identifications

and probabilities to all the unknown individuals. Next, I accepted as correctly identified all individuals for which the discriminant function had assigned the correct species definition with a 99% probability or greater. I then re-ran the discriminant function analysis and considered all individuals which the analysis misidentified (assigned the individual to the incorrect species) or correctly identified but with less than 99% probability as potential hybrids or aberrant individuals, or organisms which I had misidentified.

Molecular Analysis:

Treatment of Samples:

Approximately 25mg of tissue was used from each sample for DNA extraction. Tissue was minced on sterilized microscope slides using a sterile razor blade that had been run through a flame. Tissues were purified according to the Qiagen DNeasy® protocol for purification of total DNA from animal tissues. Samples were lysed with Qiagen lysis buffer and proteinase K for at least 3 hours (in some cases overnight) before continuing with the purification protocol. Elution was performed with 100 µl of buffer AE in order to maximize DNA concentration. To ensure DNA was extracted properly, samples were visualized on 1% agarose gel containing ethidium bromide.

PCR primers for a partial coding sequence of the Arginine Kinase gene including both exon and intron sections were designed based on sequences found on GenBank (Suzuki and Yamamato, 2000;) (Table 5). Primers were purchased from Integrated DNA Technologies (idtdna.com). PCR reactions were prepared using 50µl PCR master mix,

Table 5. Primers used in this study for isolating a section of the Arginine Kinase gene in *Anthopleura spp.* Designed based GenBank DNA sequences accession numbers AY531301.1 - AY531352.1.

5’ – cagtaaccgtcgacgtcttg – 3’
5’ – agtgatgaatctttgaggccttc – 3’

2µl DNA, 1µl of each primer and 46µl sterile H_2O. PCR parameters are shown in Table 6. Following PCR, samples were purified using the Fermentas PCR purification kit and standard protocols. Samples were then de-salted and concentrated using Amicon® Ultra centrifugal filters. De-salted samples were sent to Lone Star Labs (lslabs.com) for sequencing.

Screening for Sequence Reliability:

Raw DNA sequences were tested for reliability using the online GUIDANCE server with PRANK as the MSA (multiple sequence alignment) algorithm (Penn *et. al.*, 2010; Loytynoja, 2008). After an initial GUIDANCE analysis, individual DNA sequences with reliability scores below 0.946 were removed from further analysis. Three sequences, all involving *A. sola*, were eliminated for this reason. I then ran GUIDANCE on only the confidently aligned sequences once more which allowed me to remove the unreliable loci from analysis. All columns in the sequence which scored below 0.93 were removed from the data set. Sixty-seven sequences with a total length of 568 loci were retained at the conclusion of this process (Table 3). These sequences were used for phylogenetic and population genetics analyses.

Phylogenetic Approach:

Maximum parsimony and maximum likelihood trees were constructed in Mega 5® using the GUIDANCE aligned sequences. Maximum parsimony was computed using complete deletion of gaps and close-neighbor-interchange as the search method. I used the default setting of 10

Table 6. PCR parameters used for isolating DNA from *Anthopleura spp*.

		Temp. (˚C)	**Time (min)**
Initial Denaturation		95	2
Replication: 35 Cycles	Denaturation	95	1
	Annealing	54	1
	Extension	72	2
Final Extension		72	5

initial trees for random addition. Because maximum parsimony typically results in several equally parsimonious trees (79 in this case), I computed a consensus tree with a cut-off value of 50% so that any clades occurring in less than 50% of the trees would be displayed as a polytomy. I used bootstrapping as a test for phylogeny, with 2000 bootstrap replicates.

Maximum likelihood requires the use of an evolutionary model. Using Mega 5, I determined the best-fit model for this data set to be T92+G with 108 parameters, as was recommended by MEGA. T92 refers to Tamura (1992) which is a 2 parameter model which takes into account both that substitution can occur at different rates and that transitions and transversions may occur at different rates. Furthermore, this model adds a correction for compositional bias. If the ratios of bases differ from equal, it takes into account these differences. Finally, the 'G' refers to gamma distribution which assumes a 'well-behaved' distribution of rates across sites (Hall, 2011). I used close-neighbor interchange as the heuristic method and set the gaps/missing data treatment to 'partial deletion' with a 95% cutoff rate so that all sites where at least 95% of the sequences did not have a base would be ignored.

Population Genetics Approach:

Molecular systematics approaches such as maximum likelihood and maximum parsimony work best for distinguishing groups which have substantial genetic separation between them. For finer-scale detection of genetic isolation and potential crossbreeding among the species and populations I calculated analysis of molecular variance (AMOVA) using Arlequin ® resulting in the fixation index F_{ST}. An F_{ST} significantly different from zero indicates a significant barrier to gene flow among the populations tested. For this analysis, I discarded all invariant loci (for the purpose of this study I will use loci or locus to indicate a specific nucleotide position) and loci for which less than 90% of the

anemones had sequence information. This process left 134 variable loci held in common which were used for AMOVA. For this analysis I used only anemones which had been clearly identified to the correct species by the discriminant function analysis since misidentified species could strongly distort F_{ST} which left a total of 64 individuals for analysis (Table 3).

Genetic variability among all three species was tested using an Analysis of Molecular Variance (AMOVA) with Arlequin's default settings. I divided *A. elegantissima* into three regions: North of Cape Blanco (Kalaloch, Rosario Beach, Seal Rocks), between Cape Blanco and Point Conception (Bodega Bay, Moss Landing, San Simeon), and South of Point Conception (Dana Point) in order to determine if there was any variation by region and if Point Conception or Cape Blanco were playing important roles as barriers to gene flow. *A. sola* was divided into Northern (San Simeon, Moss Landing and Bodega Bay) and Southern (Dana Point) populations to look for restriction in gene flow based on geographical separation and Point Conception. The same was also done to *A. xanthogrammica* using a Northern (Yaquina Head/Seal Rocks and Kalaloch) and Southern (Moss Landing and Bodega Bay) grouping separated by Cape Blanco. Finally, I compared each species to the others in order to look at the genetic isolation among the species.

Differences in allele frequency

To test for differences in allele frequency among the species, all 134 variable loci in 64 total individuals were examined (Table 3). Fixed differences which occurred at any

locus were noted. Within each species, any polymorphic locus was noted, and the number of each of the possible nucleotides or gaps at the locus was counted for each species. I then performed a chi square analysis on these records in order to determine if the base pair ratios of *A.elegantissima* compared to *A.sola* differed from what would be expected within normal variation.

RESULTS

Morphological analysis:

Morphological Features:

The three species of anemones varied in their range of features, but in many cases these ranges had strong overlaps among the species (Table 4). I could find no consistent changes in the range of features within a species based on latitude or site for any of the species.

Discriminant Function Analysis:

Most of the anemones could be assigned to a given species with at least 99% confidence based on Discriminant Function Analysis (Table 7). When all these individuals were included as known species, 93% [151/162] of the total set of anemones could be correctly identified by discriminant function analysis (Figure 6). *A. xanthogrammica* in general was widely divergent from the other two species based on discriminant functions 1 and 2. *A. elegantissima* and *A. sola*, while clearly clustering differently, were closer together and had more individuals with morphological overlap or ambiguous species determination (Figure 6). Only 7% [11/162] of the anemones were misidentified or correctly identified but with low confidence (Table 7). These individuals, which could be potential hybrids, are described in Table 8 and shown in Appendix A. Of particular interest in this group are the 7 individuals which

Table 7. Accuracy of identification of individuals to species by Discriminant Function Analysis. Numbers are counts. Numbers of anemones under 'Used for Calibration' are the number of anemones at calibration sites for which identification was considered certain. Those in the '99% confidence' column were identified by DFA based on the calibration individuals. Those in other columns were either identified correctly with less than 99% confidence or were identified incorrectly by DFA.

	Used for Calibration	**Correctly identified by DFA with 99%+ Confidence**	**Uncertain Identification**	**Incorrect Identification**
Anthopleura elegantissima	10	63	4	6
Anthopleura sola	20	47	1	2
Anthopleura xanthogrammica	10	36	0	3

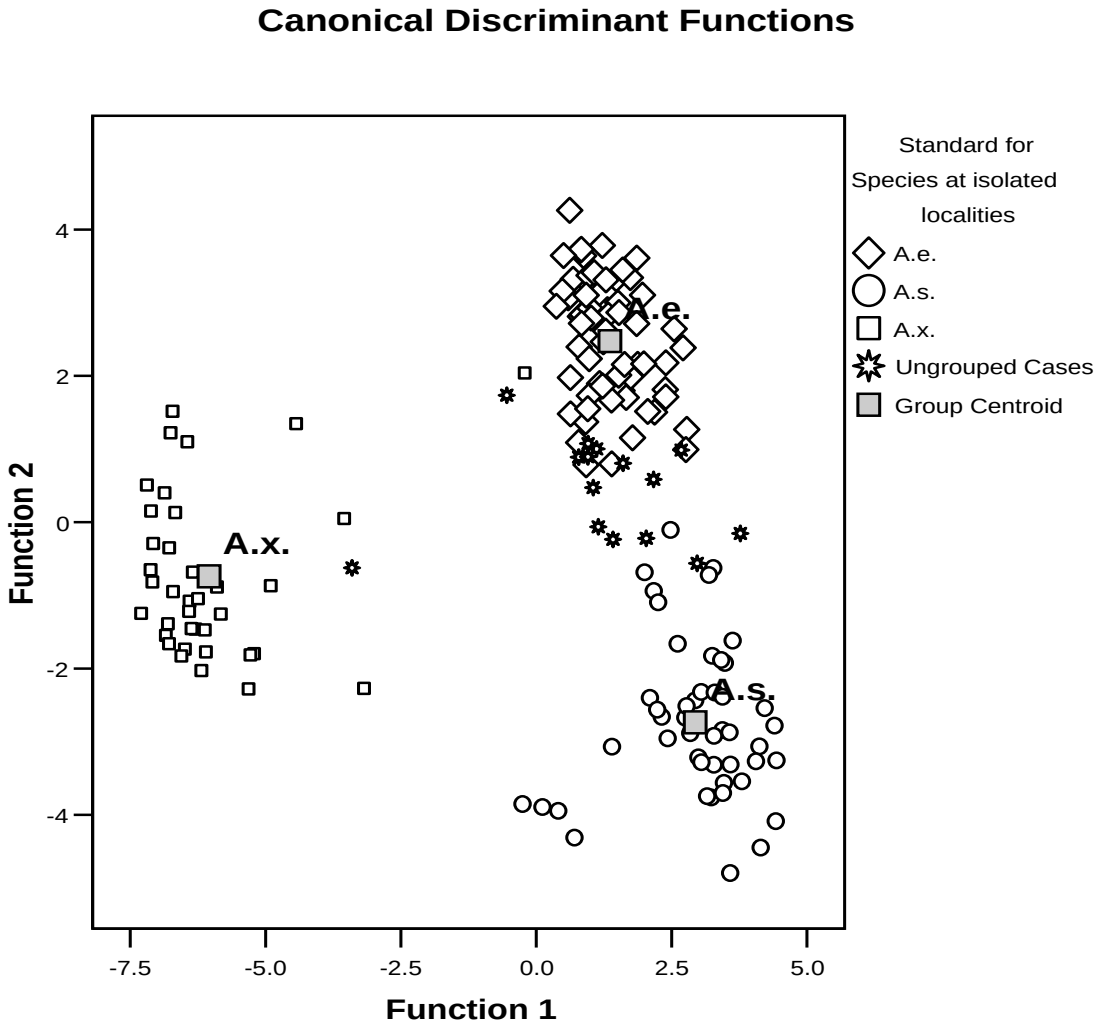

Figure 6. Separation of the three species by discriminant functions 1 and 2, based on morphological features. Most individuals could be clearly assigned to a correct species by discriminant function analysis but several uncertain identifications or misidentifications remained.

Table 8. Morphological features of anemones not easily distinguished or misidentified by discriminant function analysis. These individuals could be potential hybrids. Error code M= Misidentified by discriminant function analysis, U= identified with less than 99% confidence by discrimanant function analysis, F= confusing identification in the field. Photographs follow this table.

Error Code	Site	Species	Species assigned by Discriminant function	Figure #	Oral Disc Diameter (cm)	Oral Disc Pattern	Tentacle Length (mm)	Tentacle Width (mm)	Tentacle Length:Width Ratio	Tentacle Base Color	Tentacle Tip Color	Column Height (cm)	Column Color	Debris Cover (%)	Verrucae Pattern	Verrucae Simple or Compound	Lip Exposure	Foot Spread (mm)
M	7	A.e.	A.s.	A1 (AeSSp1)	27	1	9	1	9	2	3	9	3	40	3	1	1	2
M	7	A.e.	A.s.	A1 (AeSSp2)	40	1	13	2	6.5	3	3	10	3	50	3	1	1	8
U	7	A.e.	A.e.	A2 (AeSSp3)	13	1	6	1	6.0	3	3	7	3	90	3	1	2	2
U	7	A.e.	A.e.	A2 (AeSSp4)	17	1	5	1	5.0	3	3	9	3	20	3	1	1	3
U	7	A.e.	A.e.	A3 (AeSSp5)	22	1	12	1.5	8.0	3	3	9	3	40	3	1	1	9
M	7	A.s.	A.e.	A3 (AsSSp1)	44	2	13	2	6.5	8	3	19	3	30	3	1	3	5
U	6	A.e.	A.e.	A4 (AeMLp2)	25	1	5	1	5.0	1	1	10	3	20	3	1	3	4
F/M	6	A.e.?	A.s.	A4 (AeMLp1)	80	2	16.5	3	5.5	1	8	23	1	0	3	3	1	6
F	6	A.e.?	A.e.	A5 (AeMLp3)	87	1	11	2	5.5	1	8	20	3	0	3	3	3	10
M	5	A.e.	A.s.	A5 (AeBBp1)	45	2	11	2	5.5	1	1	9	3	5	3	3	1	7
M	5	A.e.	A.s.	A6 (AeBBp2)	43	2	12	1.5	8	1	9	11	3	80	3	1	1	5
M	5	A.s.	A.e.	A6 (AsBBp1)	81	2	11.5	2	5.8	1	8	16	3	60	3	3	3	0
U	5	A.s.	A.s.	A7 (AsBBp2)	87	2	21	4	5.3	1	6	31	3	50	3	3	3	7
M	3	A.e.	A.s.	A7 (AeYHp1)	29	2	8	1.5	5.3	0	8	8	0	40	3	1	1	2
F	3	A.e.?	A.e.	A8 (AxYHp1)	46	2	5	1	5.0	1	8	13	3	40	3	1	3	0
F	3	A.e.?	A.e.	A8 (AxYHp2)	44	2	10	2	5.0	1	8	17	3	20	3	3	1	7
M	3	A.x.	A.s.	A9 (AeSRp1)	93	1	17	4	4.3	3	3	140	3	0	1	3	2	0
M	3	A.x.	A.e.	A9 (AeSRp2)	33	0	6	1.5	4	3	3	14	3	5	3	1	3	10
F	3	A.x.?	A.x.	A10 (AxSRp1)	69	1	7	3	2.3	3	3	18	3	40	1	3	3	21
F	2	A.e.?	A.e.	A10 (AeKLp1)	64	2	20	4	5.0	7	8	18	2	40	3	3	3	5
F	2	A.e.?	A.e.	A11 (AeKLp2)	41	2	14	3	4.7	3	8	26	3	80	3	3	3	4
M	2	A.x.	A.e.	A11 (AxKLp1)	41	1	11	1.5	7.3	5	5	6	3	50	3	3	3	10

were identified as the wrong species with over 85% confidence by the discriminant function analysis.

Molecular Analysis:

Phylogenetic Approach:

Maximum likelihood and maximum parsimony trees generated by MEGA are shown in Figures 7 and 8 (respectively). On the maximum likelihood tree (Figure 7) numbers on branch nodes are bootstrap reliability values. The maximum parsimony tree (Figure 8) is a consensus tree based on 79 equally parsimonius trees. Branch values represent the percentage of these 79 trees in which that branch is present. Both methods generated approximately the same overall picture. Both methods clearly and distinctly separated *A. xanthogrammica* from *A. elegantissima* and *A. sola,* but they could not separate *A. sola* from *A. elegantissima,* nor could they separate individual populations of any of the species.

Population Genetics Approach:

Fs_T

F_{ST} values from comparing different groups of the anemones are shown in Table 9. There were highly significant barriers to gene flow among all 3 species. The barrier between *A. xanthogrammica* and the other two species was very high, indicating very

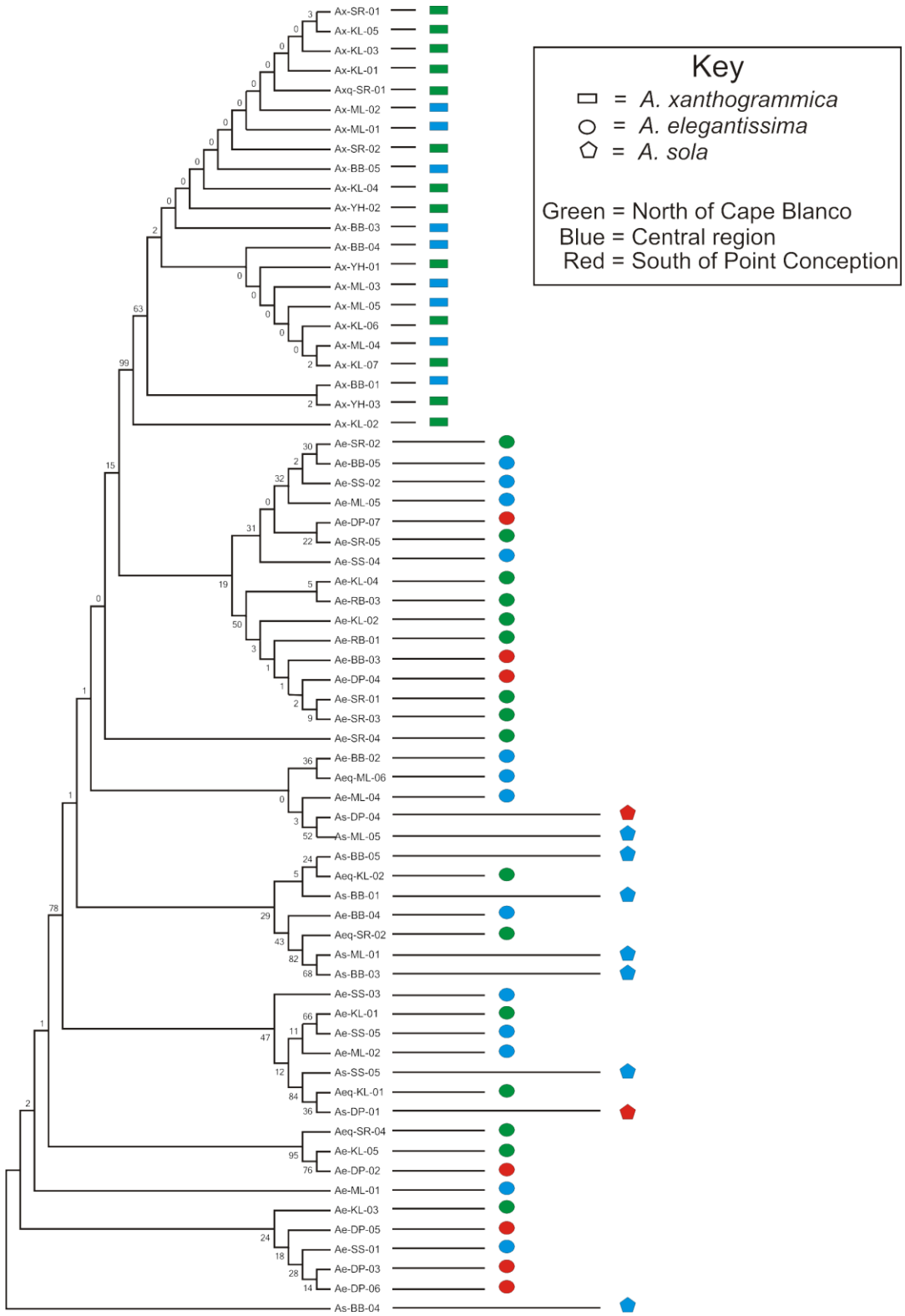

Figure 7. Maximum Likelihood tree. The numbers at each branch point represent bootstrap reliability values.

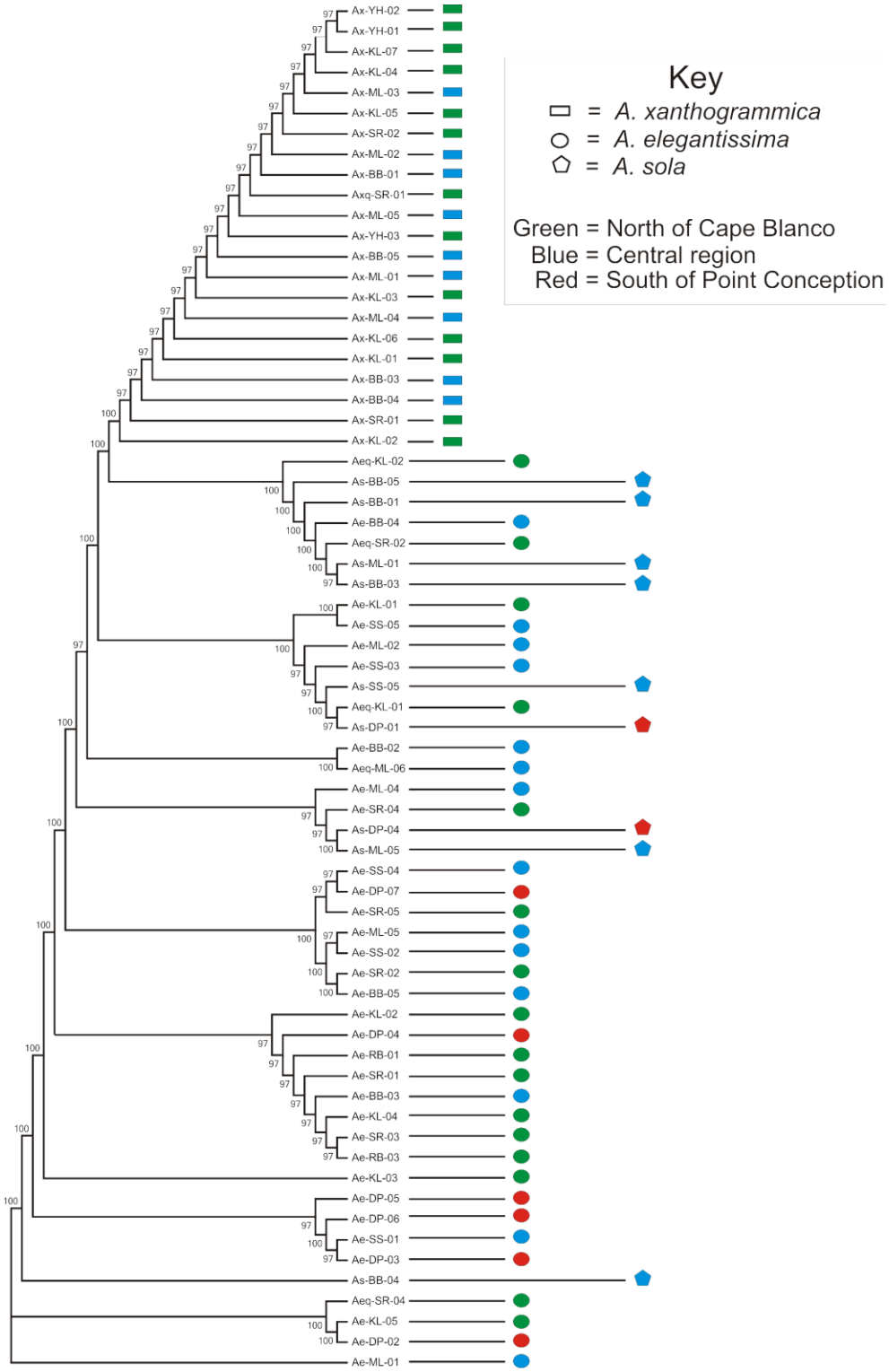

Figure 8. Maximum Parsimony tree. Numbers at branch nodes represent the percentage of the 79 equally parsimonious trees in which that branch was present.

little gene exchange among them. The barrier between *A. sola* and *A. elegantissima* was much less high though still highly significant, suggesting that very limited gene exchange may occasionally be taking place or that it may have done so in the recent past. Still, the sharply higher F_{ST} values between *A. sola* and *A. elegantissima* as compared to the F_{ST} values among populations of these species along the coast suggests that the barrier to gene exchange between the two species is so great that there is potentially greater gene exchange between individuals of the same species separated by the thousand-mile range along the coast represented by this study than between *A. sola* and *A. elegantissima* individuals in the same tide pool (Table 9). About 2/3 of the total genetic variation in a comparison of the three species was due to genetic differences among the species (Table 9). About 1/3 of the total variation was due to genetic variability within the three species. However, virtually none of this genetic variability within the species was due to genetic differences among the sites along the coast. No comparison of sites for any individual species produced anywhere near a significant F_{ST} (Table 9), suggesting that little population structure exists and that, within species, genes are being exchanged freely up and down the coast.

Differences in allele frequency

Of the 134 variable loci, there were many fixed differences between *Anthopleura xanthogrammica* and the other two species (Table 10). In sharp contrast, there were no fixed nucleotide differences between *A. elegantissima* and *A. sola*. However, Chi square analysis indicated that at 11 of the 134 loci the ratio of nucleotides was significantly

Table 9. F_{ST} values comparing different groups of *Anthopleura* anemones.

Groups	F_{ST}	Percent of Total Variation			Significance
		Within Populations	Among populations within groups	Among Groups	
All Species	0.66	34.10	0.78	65.13	0.0000
A. elegantissima vs A. sola	0.27	72.95	-0.06	27.12	0.0059
A. elegantissima vs A. xanthogrammica	0.80	19.82	0.37	79.81	0.0000
A. sola vs A. xanthogrammica	0.69	31.09	1.07	67.84	0.0000
A. elegantissima by Region[1]	0.03	97.27	-3.63	6.36	0.4057
A. sola N vs S populations[2]	-0.05	105.21	-14.01	8.79	0.7820
A. xanthogrammica N vs S populations[3]	-0.07	106.66	-2.31	-4.34	0.6569

[1]Regions for *A. elegantissima:* North of Cape Blanco, between Cape Blanco and Point Conception, South of Point Conception

[2]Regions for *A. sola*: North of Point Conception, South of Point Conception

[3]Regions for *A. xanthogrammica:* North of Cape Blanco, South of Cape Blanco

Table 10. Fixed differences between anemone species. Number of anemones involved in analysis: 31 *A. elegantissima*, 12 *A. sola*, 21 *A. xanthogrammica.*

	A. elegantissima	*A. sola*	*A. xanthogrammica*
A. elegantissima	0		
A. sola	0	0	
A. xanthogrammica	18	19	0

different between *A. elegantissima* and *A. sola*, and at another 15 loci the difference in ratio was highly significant (Table 11, Appendix C). In all, there were significantly or highly significantly different ratios of nucleotides between *A. sola* and *A. elegantissima* at 26 of the 134 variable loci, which is about 20% of the loci.

Table 11. A comparison of the polymorphism in *A. sola* (*A.s.*) to that in *A. elegantissima (A.e.)* and *A. xanthogrammica (A.x.). A. sola* being 'like' another speices means that all the nucleotides present at the locus in *A. sola* were also present in the other species. *A. sola* being 'unique' means that at least one *A. sola* had a nucleotide present at the locus that was not seen in either of the other two species.

				Monomorphic Loci			**Polymorphic Loci**		
Locus	***A.s.* like *A.e.***	***A.s.* like *A.x.***	***A.s.* unique**	***A.e.***	***A.s.***	***A.x.***	***A.e.***	***A.s.***	***A.x.***
Totals:	33	5	96	103	38	116	31	96	18
%	24.6	3.7	71.6	76.9	28.4	86.6	23.1	71.6	13.4

DISCUSSION

This study makes clear that although the three species *Anthopleura elegantissima*, *Anthopleura sola* and *Anthopleura xanthogrammica* are closely related, most individuals can be reliably distinguished to species both morphologically and genetically at all points along their range. The distinction of *A. xanthogrammica* from the other species is obvious, while that between *A. sola* and *A. elegantissima* is less distinct. The individual populations of each species, on the other hand, show signs of strong gene flow among them and show little if any subdivision to their genetic structure.

Discriminant function analysis based on morphological features mirrors that trend and reaffirms the relative difficulty of distinguishing some *A. sola* from *A. elegantissima*. In the scatterplot of morphological discriminant functions 1 and 2 (Figure 6), most *A. xanthogrammica* are clearly distinct from the other species. While *A. sola* and *A. elegantissima* definitely cluster separately from one another there is more overlap and misidentification between those two species. The features which were most likely to cause confusion in identification of *A. elegantissima* are large oral disc, faint oral disc pattern, grey tentacle base, green tentacle tips and compound verrucae. Confusing features for *A. sola* included green or grey tentacle tips, compound verrucae and pursed or unexposed lips. *A. xanthogrammica* that were small in size, had narrow tentacles, brown or tan tentacle bases or colored tentacle tips were more commonly confused (Table 8). Note that a number of these confusing characteristics are typically associated with one of the other *Anthopleura* species which likely contributes to their misidentification. An important question which this suggests is whether this

morphological overlap is primarily a result of crossbreeding or is simply due to overlapping ranges of features characteristic of the two species. Comparisons of the clearly identified versus questionable individuals of both *A. sola* and *A. elegantissima* by F_{ST} show that the aberrant individuals do not show a significant difference in their gene pools as would be expected if the aberrant individuals contained introgressive genes from the other species. Therefore the most reasonable explanation is that these two species are naturally similar in features.

The molecular approaches tell a similar story. Maximum likelihood and maximum parsimony are relatively coarse tools best suited to distinguishing species and higher levels. Both suggest that *A. elegantissima*, *A. sola,* and *A. xanthogrammica* are very similar genetically. Moderate confidence values on the maximum likelihood tree indicate that the species are not sharply distinct from one another, at least in the Arginine Kinase gene. However, *A. xanthogrammica* is further removed from both *A. elegantissima* and *A. sola*, as both methods are able to separate out all *A. xanthogrammica* individuals with high confidence (Figures 7, 8). Neither method was able to clearly separate *A. sola* from *A. elegantissima*. Maximum likelihood lumped all these individuals into a weakly branching tree with little confidence of any divisions among them. Maximum parsimony divided the two species into several subgroups with high confidence, but the subgroups did not correspond to any meaningful subdivision by species or region and it is unclear what their significance is.

In order to distinguish species or populations which are so closely related it is necessary to use tools such as F_{ST} which subdivide on a finer scale. In this case, it is valuable to be able to analyze more closely the genetics of the organisms we sampled.

Using F_{ST} to look at population genetics, it is clear that the individual species are definitely distinct in the sense of high barriers to gene exchange among them (Table 9). When aberrant *A. elegantissima* were compared to those which were easily defined by morphological analysis, they showed no strong genetic differences. Though some *A. elegantissima* resemble *A. sola* in the field, for example by having an oral disc with a diameter greater than 3.5 cm, they are genetically distinct from *A. sola* but not from other *A. elegantissima*.

Other molecular comparisons, such as a comparison of nucleotide frequencies at the polymorphic loci, also give evidence of the relative degree of separation among these species (Tables 10, 11). Groups which are freely exchanging genes should share the same alleles and at similar frequencies. However, there were at least 17 loci at which *A. xanthogrammica* had nucleotides we did not see in either *A. sola* or *A. elegantissima*. This suggests that there are multiple fixed genetic differences between *A. xanthogrammica* and the other two species, though at least 100 individuals of each species would normally need to be sampled before this could be determined with high confidence. Fixed genetic differences are regarded as powerful evidence for the lack of interbreeding and that the groups with fixed differences are in fact separate species (Hillis *et. al.* 1996). While there were no fixed differences between *A. sola* and *A. elegantissima* in the loci I studied, the significant difference in nucleotide frequencies between the two species at 26 different loci is strong evidence that these two species also are at least largely genetically isolated from one another, although the isolation may have been recent and the differences are not yet great.

In a comparison of the polymorphic loci in all three species (Table 11), *A. sola* stands out as being unique. While it does have strong sequence similarity to both of the other species and tends to have more in common with *A. elegantissima* than with *A. xanthogrammica*, its pattern of polymorphism is clearly different from either of the other species. In more than 70% of the cases where the nucleotide frequency differs among the three species it has one or more nucleotides not seen in either of the other two species. This is despite the fact that I had less *A. sola* sequences to analyze than I did for the other two species, which would normally lead me to expect less diversity of sequences. Furthermore, *A. sola* had far more polymorphic loci than either *A. elegantissima* or *A. xanthogrammica* did (Table 11). Together, these results suggest that genetic differences in *A. sola* are not primarily due to hybridization with *A. elegantissima* or *A. xanthogrammic* but instead from other outside influences. *A. sola* is genetically unique and highly polymorphic in ways different from either *A. xanthogrammica* or *A. elegantissima*.

When looking at individual populations of anemones (Table 9), a majority of the variation within each species is due to differences among individuals at a particular site rather than to differences between sites along the coast. There is proportionally more variation between anemones of the same species in one specific locality than there is between the average characteristics of anemones which may be separated from each other by hundreds of miles. This indicates that larval dispersal is not being strongly affected by any current systems or upwelling along the coast, and that genetic exchange is taking place among the populations. This conclusion is interesting in light of the determination by Sanders and Palumbi (2011) that there is strong biogeographic structuring of the algal

symbionts of *A. elegantissima* along the California coast. However, given the fact that the same *Anthopleura* species along this coast harbors several different symbiont species with different thermal tolerances at different latitudes (LaJeunesse and Trench, 2000; Secord and Augustine, 2000; Muller-Parker et al., 2007; McBride et al., 2009), it is possible that the anemones themselves are largely panmictic while their symbionts are more restricted in range.

The most likely model which could explain these data is that all three species are distinct along their ranges. While there are strong similarities among the species both genetically and morphologically, these similarities are most likely due to recent divergence from a common ancestor rather than to continuing hybridization. However, I did not find any specific evidence of introgression among these anemones but rather only of polymorphism. By testing the original sequences for heterozygosity using different molecular techniques or using a completely different sequence it may be possible to detect evidence of introgression among these species (Hellberg *et. al.*, 2002).

Previous studies on the relationships between these species of anemones have been somewhat inconclusive, specifically with regards to *A. elegantissima* and *A. sola*. Smith and Potts (1987) first argued that the solitary and clonal variations of *A. elegantissima* were virtually identically based on electrophoresis of enzymes and should be considered a single species rather than be separated into two separate species as recommended by Francis (1979). However, in 1997 McFadden *et.al.* reproduced the study by Smith and Potts (1987) with an expanded range and sample size. They concluded that the solitary and clonal forms of *A. elegantissima* represented two separate species which were only recently isolated. In 2000, Pearse and Francis used this

information as well as their own research to justify splitting *A. elegantissima* and *A. sola* into two separate species. This seems to agree with our findings in both the morphological and genetic aspects. *A. elegantissima* and *A. sola* are similar morphologically, but are still able to be differentiated by discriminant function analysis. Phylogenetic analysis based on the Arginine Kinase gene suggests that they are difficult to differentiate on a broad level, however when comparing the anemones nucleotide by nucleotide differences between *A. elegantissima* and *A. sola* stand out more clearly. While *A. elegantissima* and *A. sola* are genetically similar, the differences between them suggest that *A. sola* is reproductively isolated and is developing its own set of unique nucleotide differences.

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Appendix A: Anemones not easily identified to species

Figure A1. AeSSp1 (top) – misidentified by Discriminant Function Analysis. AeSSp2 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A Continued.

Figure A2. AeSSp3 (top) – identified with less than 99% confidence by Discriminant Function Analysis. AeSSp4 (bottom) – identified with less than 99% confidence by Discriminant Function Analysis.

Appendix A continued.

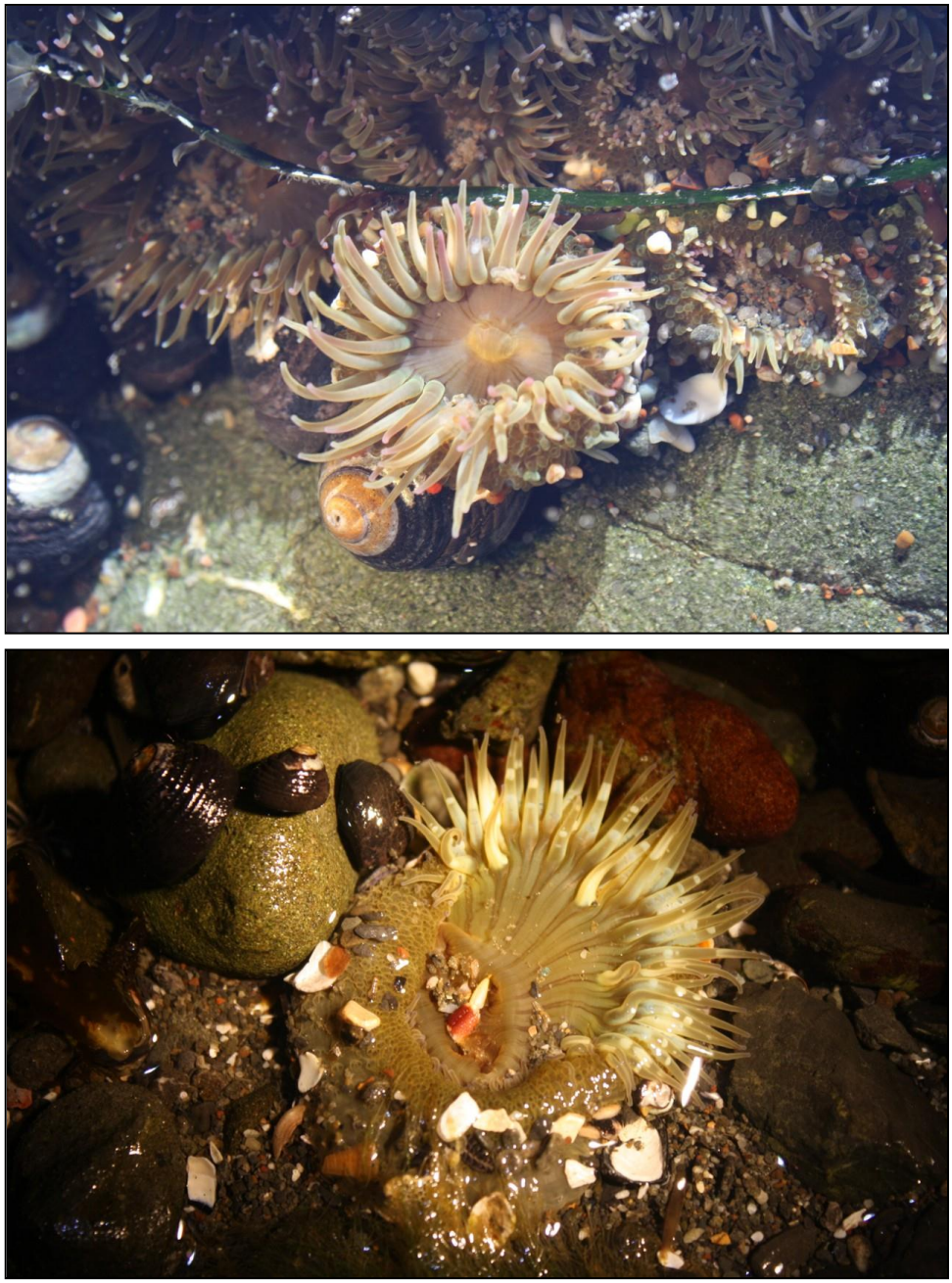

Figure A3. AeSSp5 (top) – identified with less than 99% confidence by Discriminant Function Analysis. AsSSp1 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A continued.

Figure A4. AeMLp1 (top) – misidentified by Discriminant Function Analysis and contained characteristics which were confusing in the field. AeMLp2 (bottom) – identified with less than 99% confidence by Discriminant Function Analysis.

Appendix A continued.

Figure A5. AeMLp3 (top) – contained characteristics which were confusing in the field. AeBBp1 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A continued.

Figure A6. AeBBp2 (top) – misidentified by Discriminant Function Analysis. AsBBp1 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A continued.

Figure A7. AsBBp2 (top) – identified with less than 99% confidence by Discriminant Function Analysis. AeYHp1 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A continued.

Figure A8. AxYHp1 (top) - misidentified by Discriminant Function Analysis. AxYHp2 (bottom) – misidentified by Discriminant Function Analysis.

Appendix A continued.

Figure A9. AeSRp1 (top) – contained characteristics which were confusing in the field. AeSRp2 (bottom) – contained characteristics which were confusing in the field.

Appendix A continued

Figure A10. AxSRp1 (top) – contained characteristics which were confusing in the field. AeKLp1 (bottom) – contained characteristics which were confusing in the field.

Appendix A continued.

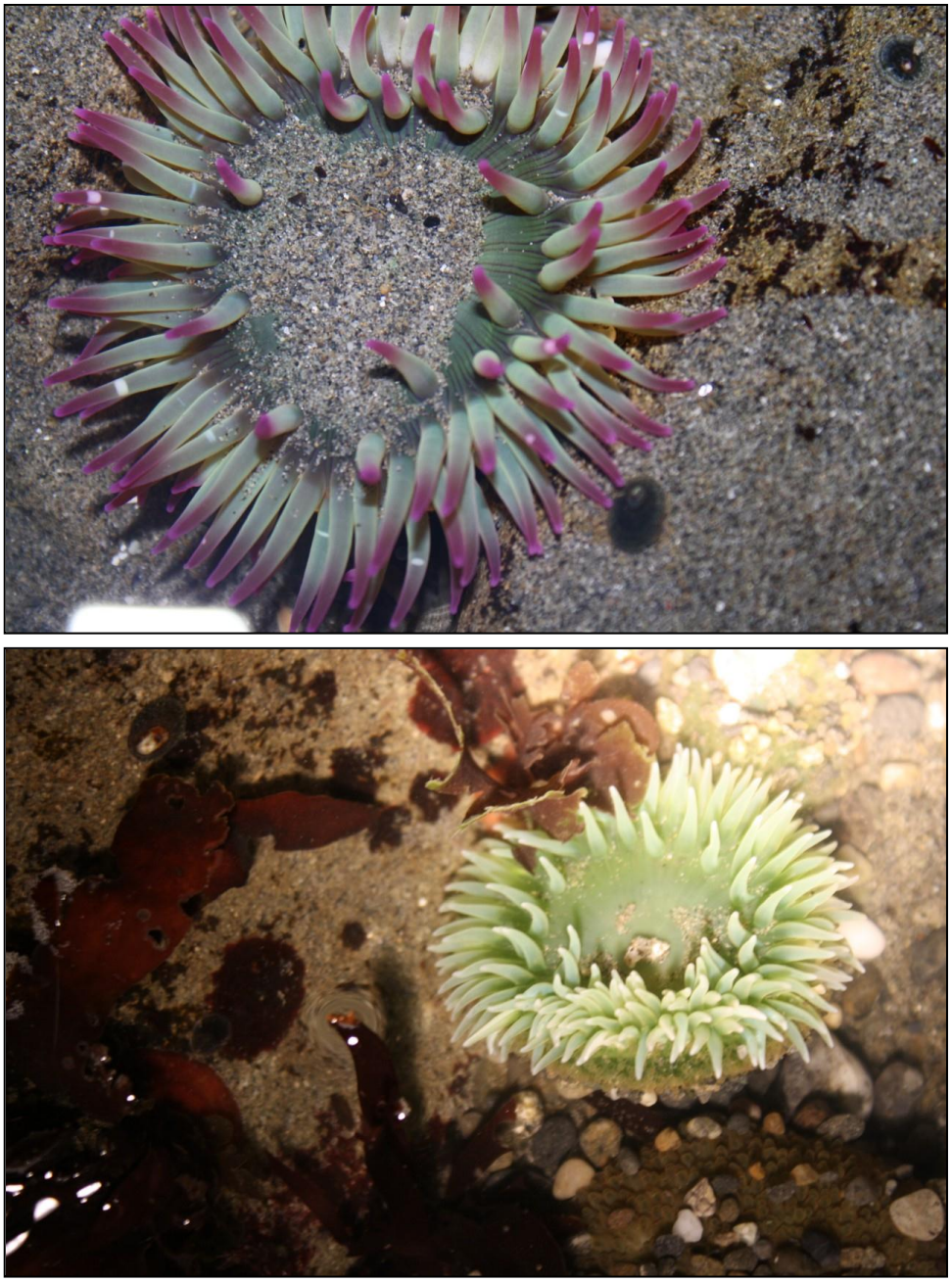

Figure A11. AeKLp2 (top) – contained characteristics which were confusing in the field. AxKLp1 (bottom) – misidentified by Discriminant Function Analysis.

Appendix B- Loci in Arginine Kinase gene used in molecular analysis

```
Loci used in this analysis.  Numbers are locus numbers from GUIDANCE align
Key:
  All lines:
   \ = a break in the sequence to trim out a section of unreliable bases
  First line:
   1 = a locus in which A. xanthogrammica has a fixed difference from A. elegantissima
but not from A. sola (none exist)
   2 = a locus in which A. xanthogrammica has a fixed difference from A. sola but not
from A. elegantissima (1 found)
   3 = a locus in which A. xanthogrammica has a fixed difference from both A.
elegantissima and A. sola (17 found)
  Second line:
   U = unvarying locus
   V = varying locus
  Lines 3-5:  Locus number, keyed to position in Guidance_align.txt (read vertically)
****************************

The sections below divide the bases into blocks of 60 for ease in printing:

Bases 472 to 644:

                      \ 3      \
            VVVVVVVVVVV \ VVVVVV \ UUUVVUUVUUUUVVUUUUUUUUUUUUUUUUUUUUUUUU
                    4   \ 5      \  6         6         6         6
                    8   \ 7      \  1         2         3         4
                    0   \ 0      \  0         0         0         0
Ae-DP-02    TGGACGAAGAG \ TACTAA \ GTGCTAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-DP-03    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-DP-05    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-DP-06    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-DP-07    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-DP-04    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SS-03    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-SS-01    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SS-02    TGGACGAAGAG \ TATTAA \ GTGCTAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SS-04    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SS-05    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-ML-04    ----------- \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-ML-01    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-ML-05    TGGACGAAGAG \ TATTAA \ GTGCTAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-ML-02    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
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Ae-BB-05    TGGACGAAGAG \ TATTAA \ GTGCTAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-BB-04    TGGACGAAGAG \ TATTAA \ GTGCCAACCTCAAACAAATTCTTGCATAAAGATTTG
Ae-SR-03    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SR-05    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SR-01    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SR-02    TGGACGAAGAG \ TATTAA \ GTGCTAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-SR-04    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-RB-03    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-RB-01    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-KL-03    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-KL-05    TGGACGAAGAG \ TACTAA \ GTGCTAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-KL-04    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Ae-KL-01    TGGACGAAGAG \ TACTAA \ GTGTCAACCTCATACAAATTCTTGCATAAAGATTTG
Ae-KL-02    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
Aeq-ML-06   TGGACGAAGAG \ TATTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
Aeq-SR-02   TGGACGAAGAG \ TACTAA \ GTGCCAACCTCAAGCAAATTCTTGCATAAAGATTTG
Aeq-SR-04   TGGACGAAGAG \ TACTAA \ GTGCTAACCTCATACAAATTCTTGCATAAAGATTTG
Aeq-KL-01   TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
Aeq-KL-02   TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTAG
As-DP-04    TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
As-DP-03    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
As-DP-01    TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
As-SS-02    AGGACGAAAAG \ TACTAA \ GTGCCAACCTCAAGCAAATTCTTGCATAAAGATTTG
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Bases 472-644 continued:

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                   8   \ 7      \  1         2         3         4
                   0   \ 0      \  0         0         0         0

As-SS-05   TGGACGAAGAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
As-ML-05   TGGACGAAGAG \ TATTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
As-ML-01   TGGACGAAGAG \ TACTAA \ GTGCCAACCTCAAGCAAATTCTTGCATAAAGATTTG
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As-BB-01   TGGACGAACAG \ TACTAA \ GTGCCAACCTCATACAAATTCTTGCATAAAGATTTG
As-BB-05   TGGACGAACAG \ TACTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
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Axq-SR-01  TGGACGAAGAG \ AACTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
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Ax-KL-07   TGGACGAAGAG \ AACTAA \ GTGCCAATCTCATACAAATTCTTGCATAAAGATTTG
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Bases 645 to 704:

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Ax-ML-02   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-ML-05   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
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Ax-BB-03   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-BB-01   ACAATTTTCACTCAATGTGATGCATCTTAAAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-BB-04   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-YH-03   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-YH-02   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-YH-01   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
Ax-SR-01   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
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Bases 645 to 704 continued:

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Ax-KL-04   ACAATTTTCACTCAATGTGATGCATCTTAGAGAAATCCCATGTAAGCTGCAATTCTATTC
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Bases 705 to 764:

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                1         2         3         4         5         6
                0         0         0         0         0         0
Ae-DP-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-DP-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-DP-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-DP-06   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-DP-07   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-DP-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SS-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-SS-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SS-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SS-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SS-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-ML-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-ML-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-ML-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-ML-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-BB-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-BB-02   CCCATTCGCCGTGAATCATATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-BB-05   CCCATTCGCCGTGAATCGGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ae-BB-04   CCCATTCGCCGTGAATCGGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-SR-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SR-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SR-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SR-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-SR-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-RB-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-RB-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-KL-03   CCCATTCGCCGTGAATCATATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Ae-KL-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-KL-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCAATGTTTTCCCATT
Ae-KL-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Ae-KL-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Aeq-ML-06  CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCAATGTTTTCCCATT
Aeq-SR-02  CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Aeq-SR-04  CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Aeq-KL-01  CCCATTCGCCGTGAATCATATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Aeq-KL-02  CCCATTCGCCGTGAATCGGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-DP-04   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATACAATGTTTTCCCATT
As-DP-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-DP-01   CCCATTCGCCGTGAATCATATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-SS-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-SS-05   CCCATTCGCCGTGAATCATATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-ML-05   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCAATGTTTTCCCATT
As-ML-01   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-BB-04   CCCATTCGCCGTGAATCGGATATAATCCCATTCCCTCGAATGATCCCTTGCTTGCCCATT
As-BB-03   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-BB-01   CCCATTCGCCGTGAATCGGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
As-BB-05   CCCATTCGCCGTGAATCGGATATAATCCCATTTCAAGGAATAATCCCATGTTTTCCCATT
As-BB-02   CCCATTCGCCGTGAATCAGATATAATCCCATTCCAAGGAATAATCCCATGTTTTCCCATT
Axq-SR-01  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-ML-04   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-ML-01   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-ML-02   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-ML-05   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-ML-03   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-BB-05   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-BB-03   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-BB-01   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-BB-04   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-YH-03   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-YH-02   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-YH-01   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-SR-01   CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
```

Bases 705 to 764 continued:

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Ax-SR-02  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-02  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-06  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-07  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-01  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-05  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-03  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
Ax-KL-04  CCCGTTCGCCGTGAATCAGATATAATCCCATTCCAAGTAATAATCCCATGTTTTCCCATT
```

Bases 765 to 824:

```
                  3333333333 3
           VUVVUUUVVVVVVVVVVVVUVUVUUVUUVVUVUUUUUUUUUUVVUVVVVVUVUUVUVVUUV
                7         7         7         8         8         8
                7         8         9         0         1         2
                0         0         0         0         0         0
Ae-DP-02   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-DP-03   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-DP-05   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-DP-06   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-DP-07   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-DP-04   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SS-03   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SS-01   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SS-02   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SS-04   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SS-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-ML-04   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-ML-01   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-ML-05   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-ML-02   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-BB-03   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-BB-02   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-BB-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-BB-04   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SR-03   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SR-05   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SR-01   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SR-02   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-SR-04   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-RB-03   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-RB-01   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-KL-03   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-KL-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-KL-04   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-KL-01   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ae-KL-02   TCAGACCAC----------GCTTGAGTAGCTTTTGTTTAATGACAACGCCTGCTAAAACG
Aeq-ML-06  TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Aeq-SR-02  TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Aeq-SR-04  TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Aeq-KL-01  TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Aeq-KL-02  TCATACCAC----------GCTTGAGTAATTTTTGTTTAATGACAACCCCTGCTAAAACG
As-DP-04   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-DP-03   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATAACGCCTGCTGCTACAACG
As-DP-01   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-SS-02   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-SS-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-ML-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-ML-01   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-BB-04   CCTTACCAA----------GCTGGATTAAGTATTGTTTAATGACTGACCCCGCCAGCACG
As-BB-03   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-BB-01   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-BB-05   TCAGACCAC----------GCTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
As-BB-02   TCCTACCCC----------GCTTGAGTAATTTTTGTTTAATGCCATCCCCTGCTAAAACC
Axq-SR-01  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-ML-04   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-ML-01   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-ML-02   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-ML-05   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-ML-03   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-BB-05   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-BB-03   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-BB-01   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-BB-04   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-YH-03   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-YH-02   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-YH-01   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-SR-01   TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
```

Bases 765 to 824 continued:

```
Ax-SR-02  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-02  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-06  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-07  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-01  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-05  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-03  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
Ax-KL-04  TCAGACCACTCACCATCTAGGTTGAGTAGTTTTTGTTTAATGACAACGCCTGCTAAAACG
```

Bases 825 to 885:

```
                                                 333 \
           VVVUVVVUUVVVVVVVVUVVVVUVVVUVUVUUUVVV \ VVUUUVVUVVVVVUVUVVVVUU
                8         8         8          \       8         8
                3         4         5          \       7         8
                0         0         0          \       0         0
Ae-DP-02   TAATTATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-DP-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-DP-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-DP-06   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-DP-07   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-DP-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SS-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SS-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SS-02   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SS-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SS-05   TAATTATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-ML-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-ML-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-ML-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-ML-02   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-BB-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-BB-02   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-BB-05   TAATGTTATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-BB-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SR-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SR-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SR-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SR-02   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-SR-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-RB-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-RB-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-KL-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-KL-05   TAATTATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-KL-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-KL-01   TAATTATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Ae-KL-02   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Aeq-ML-06  TAATGTTATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Aeq-SR-02  TAATTATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Aeq-SR-04  TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Aeq-KL-01  TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
Aeq-KL-02  TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-DP-04   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-DP-03   TGATGTCATGTTACTATGACTC---TTCCACT--- \ TCTGATCCGAGGATTAGAGTGA
As-DP-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-SS-02   TAATGATATCATAAGATGCCAC---TACTACT--- \ TCTGATGCCTCGCTTATTATGA
As-SS-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-ML-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-ML-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-BB-04   AACTGATATCTATCTATGTATCCACTTCTACT--- \ AATGACGCGTGACTAAGAAAGA
As-BB-03   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-BB-01   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-BB-05   TAATGATATCATAATATGTCTCAAATTCTACT--- \ GATGACACGTGGCTTAAAATGA
As-BB-02   CCATGTTATCTTAATTTCTCTCCACTTCTACT--- \ GATGACACCTGGCTTAACAAGA
Axq-SR-01  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-ML-04   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-ML-01   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-ML-02   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-ML-05   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-ML-03   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-BB-05   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-BB-03   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-BB-01   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-BB-04   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-YH-03   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-YH-02   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-YH-01   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-SR-01   TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
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Bases 825 to 885 continued:

```
Ax-SR-02  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-02  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-06  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-07  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-01  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-05  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-03  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
Ax-KL-04  TAATGATATCATAATATGTCTCAAATTCTACTCGA \ GATGACACGTGGCTTAAAATGA
```

Bases 886 to 913:

```
                    3
           VUVUUUVVVVUVVUVVVVVVUUUUVVVV
               8         9         9
               9         0         1
               0         0         0
Ae-DP-02   GAGAACGTTACTCCCAAGTTACTGCTTG
Ae-DP-03   GAGAACGTTACTCCCAAGTTACTGCTCG
Ae-DP-05   GAGAACGTTCCTCCCAAGTTACTGCTTG
Ae-DP-06   GAGAACGTTCCTCCCAAGTTACTGCTCG
Ae-DP-07   GAGAACGTTACGCCCAAGTTACTG----
Ae-DP-04   GAGAACGTTCCGCCCAAGTTACTG----
Ae-SS-03   GAGAACGTTACTCCCAAGTTACTG----
Ae-SS-01   GAGAACGTTCCTCCCAAGTTACTGCTCC
Ae-SS-02   GAGAACGTTACGCCCAAGTTACTG----
Ae-SS-04   GAGAACGTTACGCCCAAGTTACTG----
Ae-SS-05   GAGAACGTTACTCCCAAGTTACTG----
Ae-ML-04   GAGAACGTTACTCCCAAGTTACTG----
Ae-ML-01   GAGAACGTTACTCCCAAGTTACTGCTTG
Ae-ML-05   GAGAACGTTACGCCCAAGTTACTG----
Ae-ML-02   GAGAACGTTACTCCCAAGTTACTG----
Ae-BB-03   GAGAACGTTCCGCCCAAGTTACTG----
Ae-BB-02   AAGAACGTTACTCCCAAGTTACTG----
Ae-BB-05   GAGAACGTTACGCCCAAGTTACTG----
Ae-BB-04   GAGAACGTTACTCCCAAGTTACTG----
Ae-SR-03   GAGAACGTTCCGCCCAAGTTACTG----
Ae-SR-05   GAGAACGTTACGCCCAAGTTACTG----
Ae-SR-01   GAGAACGTTCCGCCCAAGTTACTG----
Ae-SR-02   GAGAACGTTCCGCCCAAGTTACTG----
Ae-SR-04   GAGAACGTTACTCCCAAGTTACTG----
Ae-RB-03   GAGAACGTTCCGCCCAAGTTACTG----
Ae-RB-01   GAGAACGTTCCGCCCAAGTTACTG----
Ae-KL-03   AAGAACGTTCCGCCCAAGTTACTGCTCG
Ae-KL-05   GAGAACGTTACTCCCAAGTTACTGCTTG
Ae-KL-04   GAGAACGTTCCGCCCAAGTTACTG----
Ae-KL-01   GAGAACGTTACTCCCAAGTTACTG----
Ae-KL-02   GAGAACGTTCCGCCCAAGTTACTG----
Aeq-ML-06  AAGAACGTTACTCCCAAGTTACTG----
Aeq-SR-02  GAGAACGTTACTCCCAAGTTACTG----
Aeq-SR-04  GAGAACGTTACTCCCAAGTTACTGCTTG
Aeq-KL-01  AAGAACGTTACTCCCAAGTTACTG----
Aeq-KL-02  GAGAACGTTACTCCCAAGTTACTG----
As-DP-04   GAGAACGTTACTCCCAAGTTACTG----
As-DP-03   TAGAACGATACACCCAAACTACTG----
As-DP-01   AAGAACGTTACTCCCAAGTTACTG----
As-SS-02   TAAAACAACACTCCTCCCAAACTG----
As-SS-05   AAGAACGTTACTCCCAAGTTACTG----
As-ML-05   GAGAACGTTACTCCCAAGTTACTG----
As-ML-01   GAGAACGTTACTCCCAAGTTACTG----
As-BB-04   AAGAACCTCCCTCCCAA---ACTGCTCG
As-BB-03   GAGAACGTTACTCCCAAGTTACTG----
As-BB-01   GAGAACGTTACTCCCAAGTTACTG----
As-BB-05   GAGAACGTTACTCCCAAGTTACTG----
As-BB-02   GAAAACTTTACCCCGAAGTTACTG----
Axq-SR-01  GAGAACGTTACGACCAAGTTACTG----
Ax-ML-04   GAGAACGTTACGACCAAGTTACTG----
Ax-ML-01   GAGAACGTTACGACCAAGTTACTG----
Ax-ML-02   GAGAACGTTACGACCAAGTTACTG----
Ax-ML-05   GAGAACGTTACGACCAAGTTACTG----
Ax-ML-03   GAGAACGTTACGACCAAGTTACTG----
Ax-BB-05   GAGAACGTTACGACCAAGTTACTG----
Ax-BB-03   GAGAACGTTACGACCAAGTTACTG----
Ax-BB-01   GAGAACGTTACGACCAAGTTACTG----
Ax-BB-04   GAGAACGTTACGACCAAGTTACTG----
Ax-YH-03   GAGAACGTTACGACCAAGTTACTG----
Ax-YH-02   GAGAACGTTACGACCAAGTTACTG----
Ax-YH-01   GAGAACGTTACGACCAAGTTACTG----
Ax-SR-01   GAGAACGTTACGACCAAGTTACTG----
```

Bases 886 to 913 continued:

```
Ax-SR-02  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-02  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-06  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-07  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-01  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-05  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-03  GAGAACGTTACGACCAAGTTACTG----
Ax-KL-04  GAGAACGTTACGACCAAGTTACTG----
```

Appendix C – Chi Square Analysis of Variable Loci in Arginine Kinase Gene.

Total variable loci examined in this analysis:	134
Loci at which A eleg & A sola had significantly different nucleotide ratios (p<0.05):	11
Loci at which the difference in nucleotide ratios were highly significantly different (p<0.01):	15
Total number of loci in which the ratios were significantly different:	26

Only two nucleotide variations present:

		--Counts---						
Locus		T	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
572	A eleg	24	7	31	1	13.35734	Yes	Yes
	AeExp	18.74	12.26					
	A sola	2	10	12				
	AsExp	7.26	4.74					
	Total	26	17	43				

		--Counts---						
Locus		T	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
613	A eleg	6	25	31	1	2.699215	No	No
	AeExp	4.33	26.67					
	A sola	0	12	12				
	AsExp	1.67	10.33					
	Total	6	37	43				

		--Counts---						
Locus		T	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
616	A eleg	23	8	31	1	8.75801	Yes	Yes
	AeExp	18.74	12.26					
	A sola	3	9	12				
	AsExp	7.26	4.74					
	Total	26	17	43				

		--Counts---						
Locus		A	T	Total	df	Chi Square	Signif 0.05	Signif 0.01
621	A eleg	1	30	31	1	10.64743	Yes	Yes
	AeExp	4.33	26.67					
	A sola	5	7	12				
	AsExp	1.67	10.33					
	Total	6	37	43				

Locus		--Counts--- A	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
622	A eleg	31	0	31	1	11.39316	Yes	Yes
	AeExp	28.12	2.88					
	A sola	8	4	12				
	AsExp	10.88	1.12					
	Total	39	4	43				

Locus		--Counts--- A	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
659	A eleg	1	30	31	1	21.03915	Yes	Yes
	AeExp	6.49	24.51					
	A sola	8	4	12				
	AsExp	2.51	9.49					
	Total	9	34	43				

Locus		--Counts--- T	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
662	A eleg	1	30	31	1	21.03915	Yes	Yes
	AeExp	6.49	24.51					
	A sola	8	4	12				
	AsExp	2.51	9.49					
	Total	9	34	43				

Locus		--Counts--- A	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
673	A eleg	21	10	31	1	5.043988	Yes	No
	AeExp	23.79	7.21					
	A sola	12	0	12				
	AsExp	9.21	2.79					
	Total	33	10	43				

Locus		--Counts--- A	T	Total	df	Chi Square	Signif 0.05	Signif 0.01
684	A eleg	23	8	31	1	11.76361	Yes	Yes
	AeExp	18.02	12.98					
	A sola	2	10	12				
	AsExp	6.98	5.02					
	Total	25	18	43				

Locus		--Counts--- A	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
722	A eleg	29	2	31	1	2.896477	No	No
	AeExp	27.40	3.60					
	A sola	9	3	12				
	AsExp	10.60	1.40					
	Total	38	5	43				

Locus		--Counts--- T	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
723	A eleg	2	29	31	1	1.069961	No	No
	AeExp	2.88	28.12					
	A sola	2	10	12				
	AsExp	1.12	10.88					
	Total	4	39	43				

Locus		--Counts--- T	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
742	A eleg	22	9	31	1	17.43779	Yes	Yes
	AeExp	15.86	15.14					
	A sola	0	12	12				
	AsExp	6.14	5.86					
	Total	22	21	43				

Locus		--Counts--- A	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
751	A eleg	22	9	31	1	10.3434	Yes	Yes
	AeExp	17.30	13.70					
	A sola	2	10	12				
	AsExp	6.70	5.30					
	Total	24	19	43				

Locus		--Counts--- T	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
829	A eleg	4	27	31	1	1.707196	No	No
	AeExp	2.88	28.12					
	A sola	0	12	12				
	AsExp	1.12	10.88					
	Total	4	39	43				

Locus		--Counts--- A	T	Total	df	Chi Square	Signif 0.05	Signif 0.01
835	A eleg	31	0	31	1	8.33125	Yes	Yes
	AeExp	28.84	2.16					
	A sola	9	3	12				
	AsExp	11.16	0.84					
	Total	40	3	43				

Locus		--Counts--- A	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
895	A eleg	18	13	31	1	4.448561	Yes	No
	AeExp	20.91	10.09					
	A sola	11	1	12				
	AsExp	8.09	3.91					
	Total	29	14	43				

Locus		--Counts--- C	gap	Total	df	Chi Square	Signif 0.05	Signif 0.01
910	A eleg	8	23	31	1	1.595992	No	No
	AeExp	6.49	24.51					
	A sola	1	11	12				
	AsExp	2.51	9.49					
	Total	9	34	43				

Three nucleotide variations present:

Locus		-------Counts-------- A	T	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
652	A eleg	0	30	1	31	2	12.30424	Yes	Yes
	AeExp	2.88	26.67	1.44					
	A sola	4	7	1	12				
	AsExp	1.12	10.33	0.56					
	Total	4	37	2	43				

Locus		-------Counts-------- A	T	C	Total	df	Chi Square	Signif 0.05	Signif 0.01
767	A eleg	31	0	0	31	2	5.418699	No	No
	AeExp	29.56	0.72	0.72					
	A sola	10	1	1	12				
	AsExp	11.44	0.28	0.28					
	Total	41	1	1	43				

Locus		-------Counts-------- T	C	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
794	A eleg	13	18	0	31	2	13.38452	Yes	Yes
	AeExp	17.30	12.98	0.72					
	A sola	11	0	1	12				
	AsExp	6.70	5.02	0.28					
	Total	24	18	1	43				

Locus		-------Counts-------- A	T	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
809	A eleg	31	0	0	31	2	5.418699	No	No
	AeExp	29.56	0.72	0.72					
	A sola	10	1	1	12				
	AsExp	11.44	0.28	0.28					
	Total	41	1	1	43				

Locus		-------Counts-------- T	C	G	Total	df	Chi Square	Signif 0.05	Signif 0.01
812	A eleg	0	0	31	31	2	8.33125	Yes	No
	AeExp	0.72	1.44	28.84					
	A sola	1	2	9	12				
	AsExp	0.28	0.56	11.16					
	Total	1	2	40	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	C	gap	Total	df	Square	0.05	0.01
847	A eleg	31	0	0	31	2	11.39316	Yes	Yes
	AeExp	28.12	1.44	1.44					
	A sola	8	2	2	12				
	AsExp	10.88	0.56	0.56					
	Total	39	2	2	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	C	gap	Total	df	Square	0.05	0.01
849	A eleg	31	0	0	31	2	11.39316	Yes	Yes
	AeExp	28.12	1.44	1.44					
	A sola	8	2	2	12				
	AsExp	10.88	0.56	0.56					
	Total	39	2	2	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	T	G	Total	df	Square	0.05	0.01
864	A eleg	0	0	31	31	2	8.33125	Yes	No
	AeExp	0.72	1.44	28.84					
	A sola	1	2	9	12				
	AsExp	0.28	0.56	11.16					
	Total	1	2	40	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	C	G	Total	df	Square	0.05	0.01
870	A eleg	31	0	0	31	2	8.33125	Yes	No
	AeExp	28.84	0.72	1.44					
	A sola	9	1	2	12				
	AsExp	11.16	0.28	0.56					
	Total	40	1	2	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	T	G	Total	df	Square	0.05	0.01
880	A eleg	31	0	0	31	2	8.33125	Yes	No
	AeExp	28.84	0.72	1.44					
	A sola	9	1	2	12				
	AsExp	11.16	0.28	0.56					
	Total	40	1	2	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	T	G	Total	df	Square	0.05	0.01
886	A eleg	2	0	29	31	2	9.007781	Yes	No
	AeExp	3.60	1.44	25.95					
	A sola	3	2	7	12				
	AsExp	1.40	0.56	10.05					
	Total	5	2	36	43				

		-------Counts--------					Chi	Signif	Signif
Locus		A	C	G	Total	df	Square	0.05	0.01
903	A eleg	0	0	31	31	2	5.918182	No	No
	AeExp	0.74	0.74	29.52					
	A sola	1	1	9	11				
	AsExp	0.26	0.26	10.48					
	Total	1	1	40	42				

		-------Counts--------					Chi	Signif	Signif
Locus		A	T	gap	Total	df	Square	0.05	0.01
905	A eleg	0	31	0	31	2	5.418699	No	No
	AeExp	0.72	29.56	0.72					
	A sola	1	10	1	12				
	AsExp	0.28	11.44	0.28					
	Total	1	41	1	43				

		-------Counts--------					Chi	Signif	Signif
Locus		T	C	gap	Total	df	Square	0.05	0.01
912	A eleg	4	4	23	31	2	2.037808	No	No
	AeExp	2.88	3.60	24.51					
	A sola	0	1	11	12				
	AsExp	1.12	1.40	9.49					
	Total	4	5	34	43				

		-------Counts--------					Chi	Signif	Signif
Locus		C	G	gap	Total	df	Square	0.05	0.01
913	A eleg	1	7	23	31	2	1.665026	No	No
	AeExp	0.72	5.77	24.51					
	A sola	0	1	11	12				
	AsExp	0.28	2.23	9.49					
	Total	1	8	34	43				