Genetic Connectivity and Marine Protected Areas.

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Natalie Horton, Andy R. Beaumont and Michel J. Kaiser

School of Ocean Sciences, College of Natural Sciences, Bangor University

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EXECUTIVE SUMMARY

Port Erin Closed Area (Isle of Man) is a small area of the sea bed ($2km^2$) which has been closed to scallop dredging since 1989 with the aim of protecting the sea bed from the destructive dredging gear and to act as a reserve for adult scallops. It has been shown that this closed area has resulted in an increase in adult scallop density and reproductive output within the boundaries of the reserve. The aim of this study is to estimate the contribution of the adults within the reserve to the spat settlement on commercial scallop grounds around the Isle of Man. A transfer of juvenile scallops from the Isle of Skye to the Port Erin Closed area in 2003 has created an opportunity to use genetic techniques to trace the fate of spat that originate inside the protected area. This is because there is a high likelihood that the genetic signature differs between Isle of Man and Isle of Skye scallops due to their geographical and oceanographic separations. This signature is then passed on from adult scallops inside the protected area to their offspring. If the scallops inside the protected area act as a source of spat to fishing grounds in great enough numbers, this different genetic signature should be traceable on samples taken from these areas. The first stage of the study is to identify if there is a difference between these two scallop populations.

Initial testing produced usable polymerase chain reaction (PCR) product from seven out of nine published microsatellite markers. DNA from 80 Isle of Skye and 68 native Manx scallops were then genotyped for these seven markers and the results analysed to identify population differentiation. Results showed small levels of population differentiation at three out of the seven markers which may prove useful for assigning individuals from commercial scallop beds to either native Manx or Isle of Skye source populations. However failure to comply with the Hardy-Weinberg model and extremely high polymorphism (number of alleles) may create uncertainty in the accuracy of these assignments. The power of the assignment methods is also reduced by only having three markers. Power increases rapidly with greater numbers of markers. The best option for improving certainty and increasing power would be to develop new microsatellite markers by using 454 sequencing technology to sequence large section of the genome quickly and allow efficient identification of large numbers of possible markers.

INTRODUCTION

Marine Protected Areas (MPAs) can be implemented to achieve one of two goals. Firstly to conserve biodiversity and secondly as a fisheries management tool (Hastings and Botsford 2003). In the role of fisheries management, MPAs have the potential to enhance the sustainability of stocks, especially of sessile or sedentary species by protecting habitats where adults can achieve high densities. Such high densities of adults can to lead to an increase in the reproductive output which will cause eggs and larvae to "spill-over" to areas outside the reserve, thereby acting as a source for the fishing grounds.

The success of a closed area for fisheries management will depend on the level and patterns of connectivity between it and the exploited grounds. Genetic connectivity can provide information on connectivity as it relies on two contrasting factors; larval dispersal and adult migration will serve to connect populations (gene flow) and this will be shown by a homogenisation of genetic structure between populations; conversely lack of larval dispersal and adult migration will lead to random genetic drift and local adaptations causing genetic heterogeneity between populations and genetic structure will be evident. King scallops (*Pecten maximus*) are sedentary during their adult lives with their pelagic larvae spending approximately 25 days at sea (Le Pennec et al. 2003). MPAs protecting aggregations of adults from destructive fishing methods, leads to an increase in density and reproductive output (Kaiser et al. 2007) and with 25 days as pelagic larvae there is great potential for dispersal of larvae to exploited areas. There have been several studies identifying genetic structure of scallop populations (Beaumont 1982; Heipel et al. 1998; Heipel et al. 2000; Kenchington et al. 2006). However, many of these are on a large geographic scale. Looking at connectivity on a finer scale, between an MPA and neighbouring fishing grounds, there are less likely to be any genetic differences.

Port Erin is a small (2 km^2) closed marine area in the Isle of Man (IOM). It has been closed to fishing since 1989 and densities of *P. maximus* are higher inside the closed area than outside (Beukers-Stewart et al. 2005), reproductive output is also higher inside the closed area (Beukers-Stewart et al. 2005). However, the question of whether the closed area contributes to recruitment in local fishing grounds has yet to be answered. In 2003 approximately 40,000 *P. maximus* were transferred into the Port Erin closed area from the Isle of Skye (IOS) as part of a stock enhancement program. This allows identification of any difference in the genetic signature of native IOM scallops from those introduced from the IOS. If present, this genetic signature can then be tracked outside the boundaries of the closed area to allow analysis of the contribution of Port Erin to the local fishing grounds and to identify localities that act as sources and sinks of scallop larvae around the Isle of Man.

Identifying connectivity patterns can allow more accurate models of dispersal, sources and sinks to be developed, which in turn can be used for management purposes, for example planning networks of MPAs. Inputting biological and behavioural data into oceanographic models allows the particles within the model to act like larvae rather than purely passively. Understanding larval behaviour such as vertical migration and settlement habitat will be key to improving the accuracy of these models to predict larval dispersal and connectivity patterns

To date collection of samples from the two source populations, DNA extraction, microsatellite amplification and genotyping has been conducted. Analysis of the microsatellite data to identify significant differences between the two populations has commenced.

METHODS

P. maximus samples were collected from the same grow out facility in the IOS that the transferred juveniles came from. A total of 80 individuals were sourced. *P. maximus*

samples of an age greater than when the transfers took place (i.e.: 6 years or older at the beginning of 2009) from the IOM were collected from the scallop processing plant in the Isle of Man and from the annual scallop survey onboard the research vessel Prince Madog from exploited grounds adjacent to the closed area. 68 individuals were collected. The left shell of each individual was kept to allow accurate aging using annual growth rings back in the laboratory (Mason 1983).

DNA was extracted by digestion of mantle or adductor muscle tissue by proteinase K in CTAB buffer and using standard phenol-chloroform methods, re-suspended in TE buffer and stored at 4 degrees Celsius. Published microsatellite markers for *P. maximus* (Watts et al. 2005) were optimised. Seven out of the nine markers yielded scorable PCR products. Table 1 shows PCR conditions. Microsatellites were amplified for all individuals and the PCR product run on a polyacrylamide gel using Licor 4300 DNA analyzer. The software SAGA was used to score the microsatellite alleles.

Table 1. PCR conditions for microsatellite amplification of Pecten maximus. All Loci have 3 µl of Buffer, 0.075 u Taq and 6.725 µl sterile water per 15 µl reaction. Thermal –cycling : 95°C for 1 min, a x (95°C 30 s, *T*a°C 45 s, 72°C 45 s),b x (92°C 30 s, *T*a°C 45 s, 72°C 55 s) and 72°Cfor 10 min.

Loci	dNTP	mgcl	Primers	Annealing temp (Ta)	Cycles (a + b)	Label
PM4	0.2 mM	2 mM	0.5 pM	50 C	5 + 15	800
PM5	0.2 mM	2mM	0.5 pM	50 C	5 + 15	700
PM8	0.2 mM	2 mM	0.5 pM	55 C	5 + 15	700
PM11	0.2 mM	2 mM	0.5 pM	55 C	5 + 16	700
PM12	0.2 mM	2 mM	0.5 pM	55 C	5 + 15	800
PM13	0.2 mM	2 mM	0.5 pM	52 C	5 + 15	700
PM15	0.2 mM	2 mM	0.5 pM	55 C	5 + 16	800

DATA ANALYSIS

Allele frequencies were calculated using Genepop V4 (Rousset 2007). Percentage unique alleles and total unique allele frequency for each locus in each population were calculated. Genepop V4 (Rousset 2007), was used to check for concordance with the Hardy-Weinberg model and Linkage dis-equilibrium. Burn in for all analyses was set at 10,000. The batches and iterations per batch for Markov-chain algorithms were initially run at default values. However this led to low switches between sample configurations (less than 1000). Therefore batches were set at 100 and 20,000 iterations per batch to achieve high switches and improved standard errors on the p-value estimates.

Population differentiation was investigated by calculating genic differentiation in Genepop V4 (Rousset 2007) (burn-in 10,000; batches 100; iterations per batch 20,000) and the differentiation statistic D_{EST} (Jost 2008). D_{EST} is a nearly unbiased estimator of Jost's D statistic (calculated using SMOGD software (Crawford 2009)). This is considered to be more accurate for population differentiation than traditional F_{ST} and derivatives, especially for highly polymorphic data such as microsatellites. This is due to the dependence of these statistics on Hs (within population heterozygosity) which gives F_{ST} and G_{ST} an upper limit of 1-Hs (Hedrick 1999; Jost 2008; Heller and Siegismund 2009) and so with highly polymorphic loci the upper limit of F_{ST} will track towards zero. D_{EST} has a value of zero for no differentiation and a value of 1 for absolute differentiation.

RESULTS

Amplification of scorable product was variable with some non-amplification and multiple banding appearing in some samples. Percentage amplification of scorable product varied between loci and populations (Table 2) with 68% mean amplification for IOM and 76% mean amplification for IOS.

Table 2. Percentage amplification of scorable product in 7 microsatellite markers in *Pecten maximus* samples from Isle of Man and Isle of Skye.

Locus	Percentage amplification of scorable product	
	Isle of Man	Isle of Skye
PM4	59	66
PM5	60	50
PM8	85	98
PM11	76	85
PM12	66	78
PM13	54	89
PM15	74	66
Mean over loci	68	76

Allele Frequencies

Two loci show low polymorphism with allele frequencies over 0.85 in both populations (Table 3). All other loci are highly polymorphic with allele frequencies lower than 0.30 (table 3 and see appendix 1). Table 3 shows the most frequent allele, percent unique alleles and total unique allele frequency for each loci in each population. PM13 has the highest percentage unique alleles in both populations but due to many of the unique alleles having low frequencies the total unique allele frequency is higher in PM15 in both populations. PM4 and PM8 have a single allele that is extremely frequent in both populations with frequencies greater than 0.85.

Hardy-Weinberg Model

There is an excess of homozygotes at all loci, as shown by positive Fis values, in both populations except for PM4 in the Isle of Sky and PM5 in the Isle of Man. This deviation from the Hardy-Weinberg model is significant after P-value correction, using the

Hochberg method (Hochberg 1988), in five of seven loci in the Isle of Man and 4 of seven loci in the Isle of Sky (Table 4). Combined significance using Fishers Combined probabilities method (Fisher 1932) shows that both populations significantly deviate from the Hardy-Weinberg model over all loci (Critical $X^2_{(0.05, 14)}$ = 23.69, IOM Fishers' X^2 = 80.46, IOS Fishers' X^2 = 90.22).

Linkage Disequilibrium

There was no evidence for significant linkage disequilibrium following Bonferroni correction (Hochberg method (Hochberg 1988)).

Table 3. Frequent alleles, percentage unique alleles and total unique allele frequencies of *Pecten maximus* from Isle of Skye and Isle of Man.

Locus	IOM			IOS		
	Most frequent allele (frequency)	% unique alleles	Total unique allele frequency	Most frequent allele (frequency)	% unique alleles	Total unique allele frequency
PM4	301 (0.950)	3	0.025	301 (0.868)	0	0.000
PM5	260 (0.244)	31	0.098	260 (0.250)	35	0.113
PM8	180 (0.880)	40	0.043	180 (0.850)	25	0.006
PM11	290 (0.087)	38	0.310	290 (0.074)	49	0.410
PM12	184 (0.350)	20	0.056	182 (0.300)	8	0.001
PM13	380 (0.150)	43	0.297	380 / 384 (0.127)	66	0.373
PM15	238 (0.110)	42	0.350	234 (0.066)	49	0.410

Table 4. By Locus P-values for test for deviation from Hardy-Weinberg equilibrium due to heterozygote deficiency and Fis estimates (Weir and Cochran methodology) from Genepop V4 (Rousset 2008), from Pecten maximus microsatellite data from the Isle of Man (IOM) and Isle of Skye (IOS).

Locus	IOM		IOS	
	P-value	Fis	P-Value	Fis
PM4	0.013	0.49	1.000	-0.11
PM5	0.969	-0.07	0.436	0.02
PM8	0.004	0.31	0.152	0.08
PM11	<0.001	0.19	<0.001	0.19
PM12	0.149	0.14	<0.001	0.21
PM13	0.011	0.14	<0.001	0.16
PM15	<0.001	0.65	<0.001	0.58

Population Differentiation

D_{EST}, measure of population differentiation, varies considerably between loci with a harmonic mean of 0.035. It is greatest at locus PM15 (0.357) and lowest at PM5, 8 and 12 with a value of zero (table 5). Allelic differentiation between populations is highly significant over all loci but again varies considerably between loci (Table 5).

Table 5. Population differentiation statistics for Pecten maximus from Isle of Man and Isle of Skye using microsatellite markers. Allelic differentiation using Markov Chain algorithms in Genepop V4 (Roust 2008) and Population differentiation Statistic DEST (Jost 2008) are shown.

Locus	Allelic Differentiation		D_{EST}
	P-value	SE	
PM4	0.015	<0.0001	0.008
PM5	0.239	0.003	0.000
PM8	0.037	0.001	0.000
PM11	0.004	<0.0001	0.191
PM12	0.875	0.002	0.000
PM13	<0.001	<0.0001	0.192
PM15	<0.001	<0.0001	0.357
All loci / Harmonic Mean	X^2 = infinity, P = highly sig.		0.035

DISCUSSION

The percentage amplification in this study is lower than expected in most loci. This could be due to the presence of homozygote non-amplifying alleles (Null alleles). Null alleles are caused by a mutation in the primer region of the DNA. This would also explain the significant excess of homozygotes as individuals that are heterozygous for the null allele may appear as homozygotes when genotyped. However, some individuals failed to amplify for any loci suggesting a problem with the DNA for those individuals. Degradation of the DNA could lead to poor yield and a signal too weak to register on the polyacrylamide gel. Using a DNA clean-up, such as DNA wizard by Protégé, may help improve the DNA quality and amplification. Several individuals consistently amplified multiple bands suggesting contamination. Null alleles can cause deviation from the Hardy-Weinberg model of genotype frequencies. This may have been a contributing factor to the significant deficit of heterozygotes seen in this data. Hardy-Weinberg equilibrium is an assumption of many of the assignment methods (Manel et al. 2005) so deviation from the model may eliminate the use of methods that require strict adherences to this assumption.

Although five out of the seven loci had the same most common allele in both populations there was still the presence of alleles unique to each population in all population/loci. Due to extremely high polymorphism in PM11, 13 and 15 individual unique allele frequencies are quite low (See Appendix 1), however when all unique alleles are combined for a population it can be seen that the frequency of unique alleles in these three loci is high in both populations. This presence of unique alleles suggests that there is differentiation between the two populations. This is supported by the significant allelic differentiation tests and the D_{EST} statistics, again loci PM11, 13 and 15 showing greatest differences between populations. PM5 has a high frequency of unique alleles in IOS, however the population differentiation statistics do not show significant population structuring. It should also be noted that the allelic differentiation over all

loci is highly significantly different between populations. These results suggest limited gene flow between Isle of Man and Isle of Skye populations of *P. maximus*, resulting in these three loci and possibly PM5, due to its high number of unique alleles in IOS, useful in assigning individuals sampled from exploited grounds to either source population. However, PM11, PM13 and PM15 also significantly deviate from Hardy-Weinberg equilibrium which is an assumption for some assignment tests and could lead to "miss-assignment" of individuals. With only three loci showing significant differentiation the power to assign will be lower than with a greater number of loci but having high polymorphism increases assignment power (Manel et al 2005). If we assume that an individual can be successfully assigned to their source population by having a unique allele present then we can use simple probabilities to estimate the chance of successfully identifying and assigning and IOS scallop. The probability of an IOS scallop having a unique IOS allele at any one of the markers is one minus the probability of all markers having shared alleles. This is calculated by multiplying the probabilities of getting a shared allele at each individual marker.

$P_u = 1 - P_s$

$P_s = (P_{s1} \times P_{s2} \ldots\ldots \times P_{sn})$

Where P_U is the probability of getting any unique allele, P_s is the probability of getting all shared alleles and P_{s1} is the probability of getting a shared allele at marker 1, Psn is the probability of getting a shared allele at marker n.

Using the four loci with high total unique allele frequencies (PM5, PM11, PM13, PM15) we can solve this equation to get an estimation of the probability of correctly identifying and assigning an IOS scallop based on unique allele presence.

$P_u = 1 - (0.887 \times 0.59 \times 0.627 \times 0.59)$

$= 0.806$

It should be noted that this approximation of the power to assign assumes that the alleles identified in the samples is the true allele frequencies of the whole population. Error associated with the assignment can be created when alleles identified as unique to one population are actually present in the other population but not in the sample analysed. This is an area of concern for the three highly polymorphic loci that show population differentiation (PM11, PM13, PM15). For example in locus PM11 there were 52 and 68 individuals successfully genotyped in IOM and IOS respectively. Out of these there were 52 and 63 alleles identified respectively. The concern is that new alleles will continue to occur with each new individual genotyped with those alleles currently identified as unique to one of the populations eventually occurring in the other population and individuals will be mis-assigned.

Power to assign could be improved by increasing the number of loci. Several options are available to this end. Firstly another of the published microsatellite markers for *P. maximus* has been shown to produce product during PCR. However it has not been possible, so far, to optimise PCR conditions sufficiently to yield scorable product. Further optimisation trials with cleaned-up DNA and magnesium chloride and free nucleotide concentration gradients may produce a usable marker. Microsatellite markers from other scallop species have been purchased and will be amplified on a sample of *P. maximus* and visualised on agarose gel to check the possibility of cross-amplification. The best option will be to specifically design some new microsatellite markers for *P. maximus*. This can be done by sequencing a large portion of the genome using 454 sequencer technology. This is a new generation sequencer that can produce large amounts of sequenced DNA very quickly. Large numbers of microsatellites can then be identified and optimised in a relatively short time frame. Developing new markers would also be the best option for the problem of extreme polymorphism discussed previously.

Once all marker opportunities have been investigated work will commence on assigning individuals from exploited grounds to source populations to investigate if the IOS signal

can be located anywhere other than Port Erin Closed Area. This will start by extracting DNA and genotyping 300 individuals from Targets fishing ground.

Further work will include a population genetics study of *P. maximus* around Europe. This data will increase understanding of connectivity between different populations of *P. maximus* and can aid spatial planning of fisheries management. Connectivity of populations of species with a sessile adult phase relies on larval dispersal. An important and deficient area of knowledge for understanding and predicting dispersal is larval behaviour. A study will be undertaken to study the vertical migrations and settlement patterns of larval *P. maximus* around the Isle of Man.

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APPENDIX 1

RAW ALLELE FREQUENCIES

PM4	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
279	1	5	0.013	0.047
298	1	9	0.013	0.085
301	76	92	0.950	0.868
303	2	0	0.025	0.000
304	0	2	0.000	0.019
Total alleles	**4**	**4**		
Total Sample	**40**	**54**		

PM5	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
216	0	1	0.000	0.013
233	2	0	0.024	0.000
240	0	1	0.000	0.013
242	1	0	0.012	0.000
244	0	1	0.000	0.013
246	8	9	0.098	0.113
248	3	3	0.037	0.038
250	0	2	0.000	0.025
252	1	1	0.012	0.013
254	12	6	0.146	0.075
256	8	11	0.098	0.138
258	9	5	0.110	0.063
260	20	20	0.244	0.250
262	5	7	0.061	0.088
264	2	5	0.024	0.063
266	3	3	0.037	0.038
268	0	3	0.000	0.038
270	1	0	0.012	0.000
272	3	0	0.037	0.000
274	3	1	0.037	0.013
276	0	1	0.000	0.013
278	1	0	0.012	0.000
Total alleles	**16**	**17**		
Total sample	**41**	**40**		

PM8	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
180	102	133	0.879	0.853
182	3	0	0.026	0.000
187	0	1	0.000	0.006
193	4	11	0.034	0.071
195	5	11	0.043	0.071
197	2	0	0.017	0.000
Total alleles	**5**	**4**		
Total sample	**58**	**78**		

PM11	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
212	0	2	0.000	0.015
213	0	1	0.000	0.007
220	0	3	0.000	0.022
227	1	1	0.010	0.007
232	1	0	0.010	0.000
238	1	0	0.010	0.000
239	0	1	0.000	0.007
242	1	0	0.010	0.000
243	1	0	0.010	0.000
246	0	1	0.000	0.007
247	0	1	0.000	0.007
255	0	1	0.000	0.007
256	1	1	0.010	0.007
257	1	4	0.010	0.029
260	1	1	0.010	0.007
261	0	1	0.000	0.007
262	0	1	0.000	0.007
263	0	1	0.000	0.007
265	0	1	0.000	0.007
266	0	1	0.000	0.007
267	4	0	0.038	0.000
268	0	2	0.000	0.015
269	2	0	0.019	0.000
270	0	2	0.000	0.015
271	4	1	0.038	0.007
272	0	2	0.000	0.015
274	0	1	0.000	0.007
275	1	0	0.010	0.000
277	0	1	0.000	0.007
280	0	1	0.000	0.007
281	1	0	0.010	0.000
282	0	1	0.000	0.007
283	1	0	0.010	0.000
284	1	2	0.010	0.015
285	1	1	0.010	0.007
286	0	3	0.000	0.022

289	3	6	0.029	0.044
290	9	10	0.087	0.074
291	2	1	0.019	0.007
292	6	1	0.058	0.007
293	1	2	0.010	0.015
294	2	4	0.019	0.029
295	0	8	0.000	0.059
296	3	7	0.029	0.051
297	1	1	0.010	0.007
298	4	1	0.038	0.007
299	2	0	0.019	0.000
301	2	1	0.019	0.007
302	2	3	0.019	0.022
303	1	2	0.010	0.015
304	4	5	0.038	0.037
305	1	2	0.010	0.015
306	2	4	0.019	0.029
307	0	3	0.000	0.022
308	3	0	0.029	0.000
309	1	2	0.010	0.015
310	3	5	0.029	0.037
311	2	2	0.019	0.015
312	0	1	0.000	0.007
313	0	6	0.000	0.044
314	1	3	0.010	0.022
315	0	2	0.000	0.015
316	1	1	0.010	0.007
317	1	1	0.010	0.007
318	2	0	0.019	0.000
320	0	1	0.000	0.007
321	1	0	0.010	0.000
322	0	1	0.000	0.007
325	2	0	0.019	0.000
326	2	0	0.019	0.000
328	1	0	0.010	0.000
330	0	1	0.000	0.007
332	1	1	0.010	0.007
334	0	3	0.000	0.022
335	1	1	0.010	0.007
336	2	0	0.019	0.000
338	7	2	0.067	0.015
340	2	0	0.019	0.000
341	0	1	0.000	0.007
346	1	1	0.010	0.007
348	1	0	0.010	0.000
349	1	0	0.010	0.000
374	0	1	0.000	0.007
Total alleles	**52**	**63**		
Total sample	**52**	**68**		

PM12	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
168	1	0	0.019	0.000
170	1	1	0.019	0.008
172	1	1	0.019	0.008
174	0	1	0.000	0.008
176	1	1	0.019	0.008
178	3	4	0.056	0.032
180	4	11	0.074	0.089
182	20	37	0.037	0.298
184	19	23	0.352	0.185
186	20	19	0.037	0.153
188	7	13	0.130	0.105
190	5	7	0.093	0.056
192	4	2	0.074	0.016
194	2	4	0.037	0.032
196	1	0	0.019	0.000
198	1	0	0.019	0.000
Total alleles	**15**	**13**		
Total sample	**45**	**62**		

PM13	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
264	0	1	0.000	0.007
266	0	1	0.000	0.007
288	0	2	0.000	0.014
296	2	1	0.027	0.007
300	4	2	0.054	0.014
302	0	1	0.000	0.007
303	0	2	0.000	0.014
307	10	13	0.135	0.092
311	6	0	0.081	0.000
323	1	0	0.014	0.000
326	1	1	0.014	0.007
335	0	2	0.000	0.014
344	0	1	0.000	0.007
350	0	1	0.000	0.007
355	2	0	0.027	0.000
356	1	2	0.014	0.014
360	0	2	0.000	0.014
363	1	0	0.014	0.000
364	0	1	0.000	0.007
366	0	1	0.000	0.007
367	1	0	0.014	0.000
368	0	2	0.000	0.014
372	2	1	0.027	0.007
375	0	1	0.000	0.007
376	0	2	0.000	0.014
377	0	1	0.000	0.007
378	3	12	0.041	0.085

379	1	1	0.014	0.007
380	11	18	0.149	0.127
381	0	4	0.000	0.028
382	8	2	0.108	0.014
383	1	0	0.014	0.000
384	3	18	0.041	0.127
386	0	2	0.000	0.014
387	0	3	0.000	0.021
388	1	2	0.014	0.014
392	0	2	0.000	0.014
394	0	1	0.000	0.007
396	0	1	0.000	0.007
398	3	0	0.041	0.000
399	1	0	0.014	0.000
400	1	5	0.014	0.035
402	1	4	0.014	0.028
404	1	4	0.014	0.028
405	1	0	0.014	0.000
406	0	2	0.000	0.014
407	0	1	0.000	0.007
408	1	1	0.014	0.007
409	1	0	0.014	0.000
410	1	2	0.014	0.014
411	0	3	0.000	0.021
412	0	3	0.000	0.021
415	0	2	0.000	0.014
416	0	1	0.000	0.007
420	0	1	0.000	0.007
421	0	2	0.000	0.014
422	0	1	0.000	0.007
423	2	0	0.027	0.000
424	1	0	0.014	0.000
430	0	1	0.000	0.007
434	1	0	0.014	0.000
442	0	1	0.000	0.007
458	0	1	0.000	0.007
Total alleles	**30**	**50**		
Total sample	**37**	**71**		

PM15	Count		Frequency	
Allele	IOM	IOS	IOM	IOS
144	0	3	0.000	0.028
186	1	0	0.010	0.000
190	0	1	0.000	0.009
192	3	4	0.030	0.038
193	2	0	0.020	0.000
194	5	0	0.050	0.000
196	3	0	0.030	0.000
200	1	0	0.010	0.000
202	0	1	0.000	0.009
203	4	2	0.040	0.019
204	1	3	0.010	0.028
205	2	0	0.020	0.000
206	3	1	0.030	0.009
207	2	1	0.020	0.009
208	0	2	0.000	0.019
210	3	2	0.030	0.019
211	0	2	0.000	0.019
212	2	5	0.020	0.047
213	0	5	0.000	0.047
214	1	2	0.010	0.019
215	2	3	0.020	0.028
216	1	3	0.010	0.028
217	0	5	0.000	0.047
218	0	1	0.000	0.009
219	1	0	0.010	0.000
220	3	4	0.030	0.038
221	0	2	0.000	0.019
222	4	3	0.040	0.028
224	3	3	0.030	0.028
226	3	2	0.030	0.019
227	1	0	0.010	0.000
230	5	3	0.050	0.028
232	3	3	0.030	0.028
233	2	0	0.020	0.000
234	2	7	0.020	0.066
236	0	5	0.000	0.047
238	11	1	0.110	0.009
240	4	6	0.040	0.057
241	2	0	0.020	0.000
242	0	2	0.000	0.019
244	2	1	0.020	0.009
245	0	2	0.000	0.019
246	0	1	0.000	0.009
247	1	0	0.010	0.000
248	2	2	0.020	0.019
250	4	0	0.040	0.000
252	0	2	0.000	0.019
254	4	0	0.040	0.000

258	0	1	0.000	0.009
260	1	2	0.010	0.019
263	0	2	0.000	0.019
269	0	2	0.000	0.019
272	2	0	0.020	0.000
273	0	1	0.000	0.009
278	0	1	0.000	0.009
280	0	1	0.000	0.009
290	2	0	0.020	0.000
298	2	0	0.020	0.000
319	0	1	0.000	0.009
Total allele	**38**	**43**		
Total sample	**50**	**53**		