Development of Conservation Strategies for the Critically Endangered Mekong Giant Catfish, *Pangasianodon gigas*

Conservation of genetic resources of captive stock

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Foreword

As part of conservation efforts to the critically endangered Mekong giant catfish (MGC), a survey on genetic variation using mitochondrial DNA (mtDNA) sequences and microsatellite loci was undertaken based on 259 individuals of MGC amongst seven hatcheries in Thailand and a breeding strategy was developed based on this data.

Overall, levels of genetic variation of MGC in captive stock is commensurate to that observed in the wild population. Six different breeding plans were proposed, and a simulation program was used to generate their offspring accordingly. All plans generated high levels of genetic variation, with no significant difference in the next generation. However, one plan (MP5) appeared to be best as it resulted in the highest haplotype diversity and microsatellite variation.

A number of recommendations were suggested and these are the views of the project team. We would appreciate other comments, suggestions and view points which aim to develop the best practices to conserve this important species of fish.

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1. Background

The Mekong giant catfish, *Pangasianodon gigas*, is one of the largest freshwater fishes in the world. It is endemic to the Mekong River and of cultural importance to the people of the Mekong riparian countries. Its natural population has declined in recent years and the species is classified as critically endangered (IUCN, http://www.iucn.org) and listed in the Appendix 1, of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES). Factors that have contributed to the decline of natural population(s) of the Mekong giant catfish (MGC) are still unclear, and are generally attributed to a number of anthropogenic activities such as dam construction, modification of rivers for navigation and overfishing.

Various attempts have been made to conserve the MGC over the last few decades. Once of these is the success in artificial propagation of the MGC in captivity by the Department of Fisheries, Thailand in 2001 (Unakornsawat *et al.*, 2001). At first instance, mature broodstock caught during cultural ceremonies were induced for spawning, the offspring were then maintained in ponds and cultured to create a new generation of broodstock. As a result, at present there are approximately 20,000 offspring of MGC from at least 6 year-classes maintained in government and private farms throughout seven provinces of Thailand.

One objective that would assist effective conservation of MGC is to maintain a viable and genetically diverse population in captivity. It is, however, unfortunate that information with regard to levels of genetic diversity as well as genetic relationships amongst captive MGC individuals is not available. The present project therefore aimed to characterise the genetic resources of captive populations of MGC using molecular genetic markers, including sequences of the mitochondrial D-loop gene region, and microsatellite loci; the data thereby were used to develop a management plan for captive populations of MGC in Thailand, aiming to maximise genetic diversity and minimise inbreeding.

2. Materials and Methods

2.1. Sample collection

Amongst the 20,000 individuals of MGC currently maintained in captivity, there were about 260 individuals which could be used as potential broodstock. Only some individuals could be sexed. Approximately 1 cm^2 of finclip was obtained from 200 individuals and preserved in 95% ethanol until required. Relevant information on hatcheries is given in Table 1. Details on code, tag number and sex of each individual are given in Appendix 1.

The data set presented in this report includes microsatellite and mtDNA D-loop sequences of 200 said individuals, and microsatellite data collected from 59 individuals reported by Sriphairoj *et al.* (2007). In addition, mtDNA D-loop gene region of the latter was also sequenced as part of the present project.

Table 1. Hatcheries where samples of MGC were collected and analysed

Hatchery code	Hatchery name	Location	Number of samples		
			Male	Female	Unknown
AY	Ayudthaya Inland Aquaculture Research Institute	Ayudthaya province	26	27	3
CM	Chiangmai Inland Fisheries Research and Development Center	Chiangmai province	12	13	6
KS	Kalasin Inland Fisheries Station	Kalasin province	0	1	26
PL	Pitsanulok Inland Fisheries Research and Development Center	Pitsanulok	7	8	14
PY	Phayao Inland Fisheries Research and Development Center	Phayao province	24	23	32
SK	Songkhla Inland Fisheries Research and Development Center	Songkhla province	0	0	22
TK	Tak Inland Fisheries Research and Development Center	Tak province	0	0	15

2.2. Laboratory procedures

2.2.1. DNA extraction and microsatellite analysis

DNA was extracted following procedures of Taggart *et al.* (1995) with slight modification. Extracted DNA was re-suspended in TE buffer (10mM Tris-HCl pH7.5, 1mM EDTA pH8.0) until required.

A total of eight microsatellite loci was examined, of which seven (Pg-1, Pg-2, Pg-3, Pg-6, Pg-16 and Pg-17) were isolated by Na-Nakorn *et al.* (2006), and one was obtained from Hogan and May (2002). PCR mixture and condition followed Sriphairoj *et al.* (2007) and Hogan and May (2002) accordingly. PCR products were run on 4.5% denatured polyacrilamide gels and visualized by silver staining. Allele size was determined based on the M13 sequence ladder (Promega, USA).

2.2.2. mtDNA analysis

A region of the D-loop gene was amplified using primers HD (5′-GTT TAG GGG TTT GAC AGG-3′) and LD (5′-ACA CTA TTA CTG GCA TCT GG-3′) (Ngamsiri *et al*., 2007). PCR reactions were performed in a total volume of 35 µl containing 50 ng/µl of template DNA, 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1U of Taq polymerase (Promega, USA). The PCR profile comprised an initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at

94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min.

PCR products were purified and sequenced by Macrogen Inc., Korea. Both heavy and light strands were sequenced.

2.2.3. Statistical analysis

Microsatellite data were analyzed using the computer package POPGENE (Yeh and Boyle, 1997). The differences between populations were tested for each parameter (number of alleles - A, effective number of alleles - A_e, observed heterozygosity - H_o, expected heterozygosity - H_e) using ANOVA included in the SPSS statistical package (version 10) following by a post-hoc multiple comparison with Bonferroni corrected for multiple pairwise tests.

The pairwise relatedness coefficient (r_{xy}) of Ritland (1996) was adopted for this study because it gave the values best fit the theoretical relatedness coefficient values for full-sib and half-sib according to Sriphairoj *et al*. (2007). The estimation was made using the MARK computer program, written by KR in FORTRAN95 for Windows, available at http://www.genetics.forestry.ubc.ca/ritland/programs.html.

The mean kinship (*mk*) of the each individual, the average kinship values for each individual with other individuals in the population including itself, was estimated following Doyle *et al.* (2001). The mean kinship for the whole sample was calculated using Microsoft Excel.

MtDNA sequences were viewed and edited using MEGA3.1 (Kumar *et al*., 2004) and then aligned using Clustal W as implemented in the same software. Data were then imported into Arlequin version 3.1 (Schneider *et al*., 2000) for the analysis of the molecular diversity indices within populations; haplotype diversity (h) and nucleotide diversity (π) (Nei, 1987). Relationship amongst haplotypes were determined using program NETWORK (http://www.fluxus-engineering.com/sharepub.htm#a1) (Bandelt *et al*., 1999).

2.2.4. Experimental mating plans and simulations

Six different mating plans were designed as follows:

- Mating plan 1 (MP1): According to Sriphairoj *et al.* (2007) the mating scheme that involves a minimum of 20 mating pairs with the least r_{xy} could retain sufficient level of genetic variation of the stock in the next generation. Hence in MP1 we used 24 females and 24 males regardless of haplotypes, i.e. male : female = 1:1 and N_e = 48. Selection of mating pairs was based on r_{xy} values, i.e. mating pairs with the least r_{xy} will be selected to generate genotype data for the next generation.
- Mating plan 2 (MP2): In this plan, N_e was doubled compared to that in MP1, male : female ration remained 1:1. Selection of mating pairs was based on levels of microsatellite variation and number of haplotypes in the offspring.
- Mating plan 3 (MP3): All mature brooders (72 females and 69 males) were included for simulation. Mating pairs were selected mating base on the r_{xy} value, male : female ratio was 1:1 and as such there were 69 pairs in total.

- Mating plan 4 (MP4): Similar to that of MP3 with only difference is that one female could mate with more than one male provided that r_{xy} value was less than results of other mating pairs. The simulation produced matings between 72 females and 31 males.
- Mating plan 5 (MP5): This plan was designed to include all 12 haplotypes (no females were available for the haplotype 13 and 14) with equal number of representative from each haplotype. Then the pairing was made based on the least r_{xy} values with male: female ratio of 1:1. As such, the number of females representing each haplotype varied from one to three due to availability of females that represent each haplotype.
- Mating plan 6 (MP6): The plan was designed to include all 12 haplotypes but regardless of number of female individuals representing each haplotype, male : female = 1:1, pairs were then selected according to the least r_{xy} values.

Genotypes of offspring resulted from each mating plan were generated from the multilocus genotypes of the parents using the program SIMULATION COMBINE ALLELE VERSION1 (SCAV1) (Khumpeerawat pers. comm.) assuming each mating produces 200 offspring and 80% survival rate for each family. Genetic variation of the offspring generation was calculated using the program POPGENE (Yeh and Boyle, 1997). The mean r_{xy} represents inbreeding coefficient of the offspring. Therefore, advantages of the mating plans were compared based on levels of genetic variation and mean r_{xy} amongst offspring of each mating plan.

The haplotype diversity and nucleotide diversity of the expected progeny were directly estimated from the haplotypes of the female parents involved in each mating plan.

3. Results

3.1. Genetic variation in captive stocks of MGC

Microsatellite variation: Allele frequencies of each stock are shown in the Appendix 2. Across a total of 259 fish, the mean number of alleles/locus was 5.14 (±1.57) while the number of alleles/locus weighted with allele frequencies (effective number of alleles/locus) and number of alleles/locus corrected for sample size (allelic richness) were 2.80 (±0.97) and 3.93 (±1.22) respectively. The observed heterozygosity (average number of heterozygotes per locus per individual) was 0.66 (±0.12) and was in conformity to the heterozygosity estimated from Hardy-Weinberg expectation (H_e=0.61±0.12). The details of the genetic variation are shown in Table 2.

The average pairwise genetic relatedness (r_{xy}) across all samples was 0.18 (±0.01) and ranged between -0.006 and 2.412 while the r_{xy} within each stock was slightly larger [ranged from 0.20 (Ayudthaya and Phayao) to 0.40 (Songkhla)] (Table 2).

MtDNA variation: Sequences of the mtDNA D-loop gene region revealed 14 haplotypes (Table 3) across all populations among which Ayudthaya and Phayao had the highest number of haplotypes [9 and 8 haplotypes, haplotype diversities (h) = 0.817 and 0.794; nucleotide diversities (π) = 0.009 and 0.007 respectively] following by Kalasin (6 haplotypes, h=0.609, π=0.002), Chiangmai (6 haplotypes, h=0.487, π=0.005), Pitsanulok (6 haplotypes, h=0.473, π=0.006) and Songkhla (3 haplotypes; h=0.178, π=0.002). The least variable population was Taak with only one haplotype (haplotype 14, haplotype diversity = 0.000). The overall variation of mtDNA was relatively high (14 haplotypes, h=0.886, π=0.008).

Among the 14 haplotypes identified, the least abundant was haplotype 3, 6 and 8 with only two individuals per haplotype. The most abundant haplotypes was haplotype 7 (58 individuals). However at present only 128 individuals could be sexually differentiated among which 68 of them were females (Table 1). As such, haplotypes 13 and 14 were not represented in the mature females and most haplotypes were rare (e.g. haplotype 1, 3, 8, 9 and 12 were represented by only one individual each and haplotype 5 and 6 represented by 2 individuals). Relationships amongst haplotypes are presented in Figure 1.

Table 2 Genetic variation within stocks of the captive *Pangasianodon gigas* and the genetic relatedness coefficient (r_{xy}) (Ritland, 1996).

Populations	*N*	*A*	A_e	A_r	H_o	H_e	mean r_{xy}	Range of r_{xy}
1. Chiangmai	31	4.00 (1.29)	2.71 (0.94)	3.66 (1.23)	0.73 (0.19)	0.60 (0.14)	0.21 (0.10)	0.006-0.601
2. Kalasin	27	3.71 (1.38)	2.23 (0.45)	3.40 (1.13)	0.63 (0.21)	0.55 (0.09)	0.23 (0.13)	0.007-1.305
3. Tak	15	2.43 (0.53)	1.90 (0.57)	2.43 (0.53)	0.51 (0.15)	0.46 (0.14)	0.27 (0.08)	0.072-0.485
4. Phayao	79	4.00 (1.63)	2.97 (1.13)	3.78 (1.44)	0.67 (0.14)	0.62 (0.13)	0.19 (0.10)	-0.003-1.033
5. Pitsanulok	29	3.14 (1.07)	2.21 (0.49)	3.02 (0.90)	0.54 (0.13)	0.54 (0.11)	0.30 (0.17)	-0.006-0.940
6. Songkla	22	3.14 (1.57)	2.14 (0.76)	2.90 (1.35)	0.62 (0.35)	0.47 (0.25)	0.40 (0.23)	0.079-1.046
7. Ayudthaya	56	4.29 (1.11)	2.65 (0.77)	3.78 (1.08)	0.71 (0.17)	0.60 (0.11)	0.20 (0.10)	-0.006-2.412
Overall	259	5.14 (1.57)	2.80 (0.97)	3.93 (1.22)	0.66 (0.12)	0.61 (0.12)	0.18 (0.01)	-0.006-2.412

Table 3. Distribution of 14 observed mtDNA D-loop haplotypes and other diversity indices amongst hatchery samples of MGC

Haplotype	Nucleotide sites														Hatcheries							
	22	29	34	45	99	154	170	171	180	234	238	254	274	278	AY	CM	KS	PL	PY	SK	TK	Overall
1	C	G	C	C	G	A	C	T	C	C	C	G	T	T	5	1	6	1	9	1	0	23
2	.	.	.	.	.	.	.	C	.	.	.	.	.	.	7	20	2	0	29	0	0	58
3	.	.	.	.	.	.	.	.	.	.	.	.	C	.	2	0	0	0	0	0	0	2
4	.	.	.	.	.	.	.	.	.	.	T	A	.	.	0	0	0	21	0	1	0	22
5	.	.	.	.	A	.	.	.	.	.	.	.	.	.	0	0	1	0	8	0	0	9
6	.	.	.	.	A	.	.	.	.	.	.	A	.	.	1	0	0	0	1	0	0	2
7	.	.	.	T	A	.	.	.	.	.	.	A	.	.	11	1	16	1	3	0	0	32
8	.	A	.	.	.	.	.	.	.	.	.	.	.	C	2	0	0	0	0	0	0	2
9	T	.	.	.	.	.	.	.	.	.	.	.	.	C	4	0	0	0	0	0	0	4
10	.	.	T	.	A	.	T	.	T	.	.	.	.	.	19	0	2	0	0	0	0	21
11	.	A	.	.	A	.	T	.	T	.	.	.	.	.	0	3	0	3	15	0	0	21
12	.	.	.	.	A	G	T	.	T	.	.	.	.	.	0	2	0	1	2	20	0	25
13	.	.	.	.	A	.	T	.	T	T	.	.	.	.	0	0	0	0	11	0	0	11
14	.	.	.	.	A	.	T	.	T	.	.	.	.	.	4	1	0	2	1	0	15	23
No. of sample															55	28	27	29	79	22	15	255
No. of polymorphic site															11	8	7	8	9	6	0	14
No. of haplotype															9	6	5	6	9	3	1	14
Haplotype diversity (h)															0.818	0.487	0.609	0.473	0.794	0.178	0.000	0.886
±SD															(0.033)	(0.111)	(0.088)	(0.109)	(0.028)	(0.106)	(0.000)	(0.008)
Nucleotide diversity (π)															0.009	0.005	0.005	0.006	0.007	0.002	0.000	0.008
±SD															(0.005)	(0.003)	(0.003)	(0.004)	(0.004)	(0.002)	(0.000)	(0.005)

3.2. Mating plans and resulting genetic variation from simulations

Information on each mating pairs of all six mating plan are presented in Appendix 3. All mating plans resulted in appreciable genetic variation relative to mean genetic variation of the whole gene-pool (*A* ranged from 4.43 ± 1.27 to 4.71 ± 1.25, Table 4). However mean number of alleles per locus of the expected progeny (*A* ranged from 4.43 ± 1.17 to 4.71 ± 1.25) was slightly lower than the average of the parental stocks while the effective number of alleles/locus was slightly higher for the expected progeny (A_e ranged from 2.90 ± 1.06 to 3.23 ± 1.18) than the A_e of the parents. The observed heterozygosities were high relative to that of the parents following every mating plan (H_o ranged from 0.73 ± 0.14 and 0.82 ± 0.13). However the expected heterozygosities were lower than H_o (H_e ranged from 0.62± 0.13 and 0.65± 0.12, Table 4). There were no statistical differences of each genetic variation parameters between mating plans).

The differences were shown for r_{xy} wherein the lowest was from MP1 despite of non statistical significant differences with those of MP 2, MP5 and MP6. MP3 resulted in the highest r_{xy} while MP4 showed higher r_{xy} than MP1, lower than MP3 but no difference with the others (Table 4). All but one mating plan (MP1) included females representing 12 out of 14 haplotypes (no females of haplotypes 13 and 14 were available).

The MP5 which included all 12 available haplotypes in almost equal frequencies showed the highest mtDNA variation of the expecting progeny (h=0.942, π=0.009).

Table 4. Genetic diversity of offsprings resulted from simulation of six mating plans (*A* = number of allele/locus, A_e = effective number of allele/locus, H_o = observe heterozygosity, H_e = expected heterozygosity, r_{xy} = average relatedness coefficient, No. of hap. = number of represent haplotype)

Plan	N	*A*	A_e	*Ho*	H_e	r_{xy}	No. of hap.
MP1	24 females	4.57	3.21	0.82	0.65	0.022a	10
	24 males	(1.27)	(1.18)	(0.13)	(0.12)	(0.021)	
MP2	48 females	4.71	3.10	0.78	0.63	0.046ac	12
	48 males	(1.25)	(1.15)	(0.14)	(0.12)	(0.030)	
MP3	69 females	4.71 (1.25)	2.90	0.73	0.62	0.072b	12
	69 males		(1.06)	(0.14)	(0.13)	(0.050)	
MP4	72 females	4.71 (1.25)	2.99	0.78	0.63	0.047c	12
	31 males		(1.05)	(0.13)	(0.12)	(0.028)	
MP5	24 females	4.43	3.12	0.80	0.64	0.032ac	12
	24 males	(1.27)	(1.13)	(0.13)	(0.12)	(0.026)	
MP6	24 females	4.43	3.19	0.81	0.65	0.026ac	12
	24 males	(1.27)	(1.20)	(0.14)	(0.12)	(0.020)	

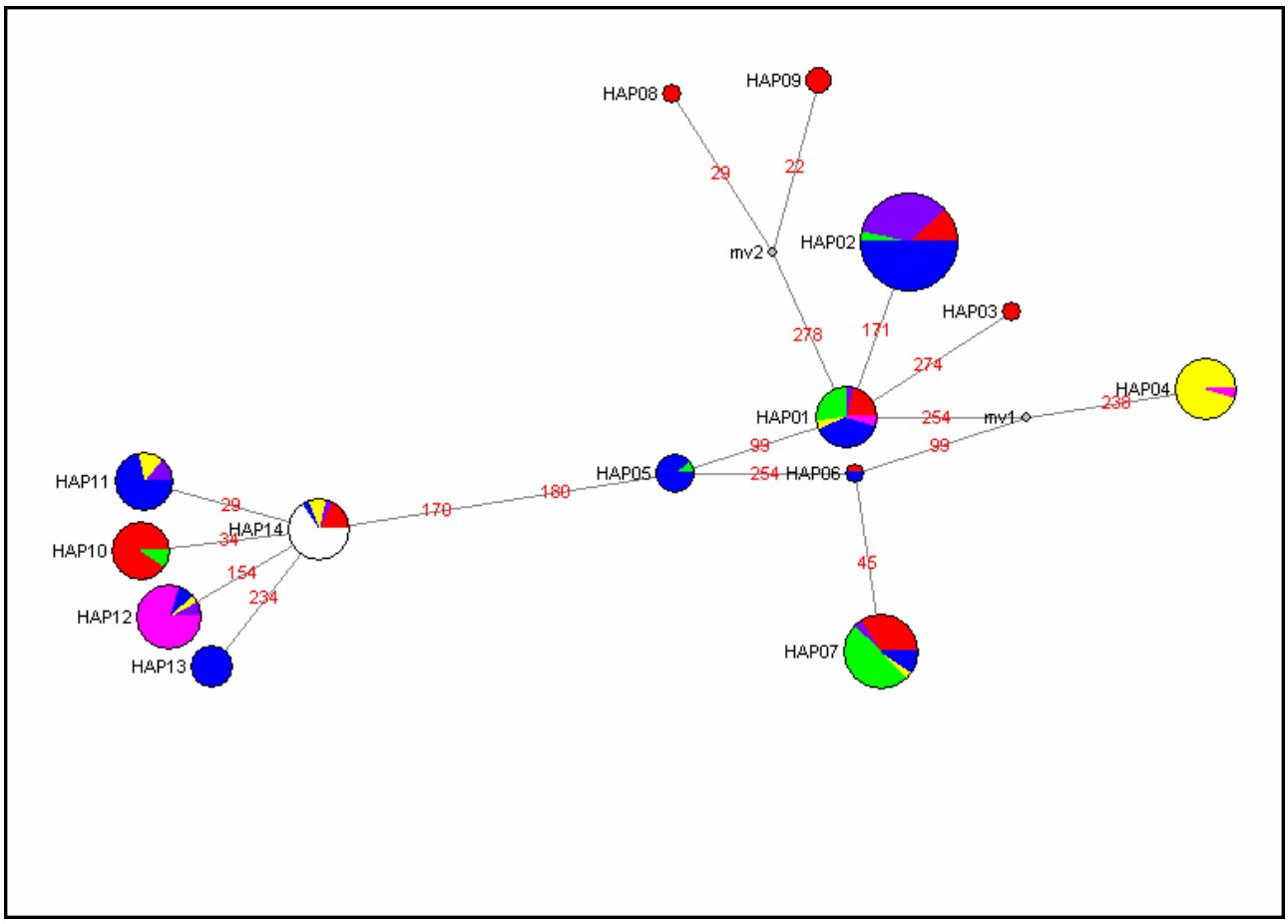

Figure 1. Network showing relationships amongst mtDNA D-loop haplotypes. Size of the circles indicate level of abundance of each haplotypes in all hatcheries. Mv1 and mv2 are missing haplotypes. Numbers in red denote sites of mutation.

4. Discussion

4.1. Level of genetic variation of captive stock of MGC

Generally loss of genetic variation in the captive stocks is a common phenomenon responsible by small effective population size (i.e. founder effects) may have been continuing until present. The consequences include loss of rare alleles accompanying by the occasional reduction of heterozygosity (Aho *et al.*, 2006; Allendorf and Phelps, 1980; Leberg, 1992; Shikano *et al.*, 2001). However, in the present study, levels of genetic variation observed in captive stock of MGC was found commensurate with that of the wild stock based on the same sets of microsatellite loci (A= 3.91 ± 2.12; A_e = 2.49 ± 1.04; H_o = 0.60 ± 0.25; H_e = 0.54 ± 0.17; n=24, Na-Nakorn *et al.*, unpublished data). This may be due to large number of founders and the brief captive history. It was also our concern that the genetic variation of the wild stock could have been underestimated due to a small sample size. Lorenzen and Sukumasavin (2007) estimated the current total spawner abundance is about 155-185 animals. As such the sample size of 24 (10%) was sufficient to represent the wild population. Therefore we are confident that the captive stock well represents the genetic diversity existed in the wild stock of Mekong giant catfish and will be useful for a conservation purpose.

4.2. Comparisons between mating plans

Simulations of all mating plans resulted in appreciable genetic variation (Table 3) relative to mean genetic variation of the whole gene-pool, a few alleles may be lost as indicated by a slightly lower mean number of alleles/locus of the expected progeny (4.43 – 4.71) than the average of the parental stocks (5.14, Appendix 2). Nevertheless relatively high effective number of alleles/locus of the expected progeny (A_e = 2.90 – 3.21 comparing to the parental A_e of 2.80) indicated even distribution of allele frequencies and hence enhance the possibility to be inherited to successive generations. Heterozygosity which is not affected by genetic drift but inbreeding (Falconer and Mackay, 1996) was higher than that of the wild populations because inbreeding was minimized through the selection of mating pairs with the least r_{xy}.

The simulated results of six mating plans gave insignificant difference in genetic variation while the difference of the mean r_{xy} of the expected offspring was significant. Therefore the best mating plan(s) should be the one with least r_{xy} and highest mt-DNA variation. The offspring of the MP1, 2, 5 and 6 showed low r_{xy} values, and there was no significant differentiation amongst them in terms r_{xy} values. MP5, however, resulted in the highest haplotype diversity. As such MP5 appeared to be best, which would produce offspring with 12 mtDNA haplotypes in total, and the lowest r_{xy} values.

5. Recommendations

1. **Number of hatcheries**: In order to avoid risks associated with unexpected catastrophes that may lead to loss of MGC broodstock, we recommend to have more than one hatchery. As can be seen from Table 2 that the four hatcheries Ayudthaya, Phayao, Chiangmai and Pitsanulok had relatively higher numbers of mtDNA haplotypes compared to others, hence these hatcheries should be considered as MGC captive gene pools.
2. **Maintaining mtDNA haplotypes**:
    a. The four hatcheries mentioned in (1) do not have all mtDNA haplotypes at present and as such these need to be brought in from other hatcheries. Females should be obtained from the nearest hatcheries if possible to avoid cost of transportation and mortality.
    b. At present the haplotype 13 and 14 do not exist in any mature females. Therefore the sexually undifferentiated individuals of these haplotypes should be inspected carefully. When individuals with haplotype 13 and 14 reached maturity and identified females they should be transferred to each of the four hatcheries assigned as the back up.
    c. Mating between female and male of the same haplotype is not recommended because they are more likely to have common ancestors than the pair with different haplotypes.
3. **Mating plan**: All individuals in the MP5 should be used to generate the next generation with the highest level of genetic variation possible at the present context.

4. **Recruitment of new broodstock**:
    a. It is generally recommended that broodstock that are used for stock enhancement purposes should come from the wild. However, it is almost impossible in the case of critically endangered species such as MGC. However this could be implemented once the natural population is fully recovered.
    b. Any new brooders should be genotypes for their genetic record.
    c. It is important to avoid impacts due to loss of adaptation to the wild environment caused by maintaining of MGC in captivity for a long period, it is important to note that F_2 individuals (from F_1 parents) will not be suitable for stock enhancement.
    d. Although the MP5 is recommended at this moment, once the wild population of MGC is recovered it is ideally that about 20% of broodstock should be replaced each year to influx of genetic material and to maintain genetic diversity, and N_e should be increased up to 100. This means for example the breeding program aims to achieve a N_e of 100 over a 10 year period then offspring from 5 breeding pairs are needed.
5. **Restocking**:
    a. Stock similar number of offspring from each of breeding pairs after at least 24 hour quarantine. Do not grade or select fish prior stocking.
    b. Link breeding program with restocking program according to plan of international and/or national authorities (i.e. 5 years, 10 years or 20 years).
6. **Research gaps**:
    a. In order to be able to sex an MGC individual it needs to be raised to reach sexual maturity. This will take about 15 years and is costly. It is therefore important to develop sex identification tools for MGC which will aid early effective selection of broodstock.
    b. It is important that male and female of each breeding pair according to MP5 need to be synchronized in terms of level of maturity. Therefore, broodstock rearing conditions (e.g. feed, controlled environments) and enhancement of gamete development by chronic application of hormone (e.g. implantation of gonadotropin releasing hormone) should be investigated to ensure that males are ready when females could spawn.
    c. In order to support (b) and to conserve genetic diversity, cryopreservation techniques for male gametes of MGC should be refined and applicable to all hatcheries. Male gametes should be cryopreserved, possibly at a central unit or share amongst hatcheries for all males in MP5. Once females are ready to spawn then cryopreserved milt of appropriate males could be used. This will reduce the risks and costs associated with transporting fish from long distance.
7. This document is developed is solely based on genetic data. A restocking program also should take into account other factors that have been attributed to the decline of MGC. A concerted effort is needed in this regard to protect this important species of fish of the world.

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Appendix 1. Sample code and relevant information such as tag number, sex and mtDNA D-loop haplotype. M = male, F = female, NA = Not known

1. Ayudthaya Inland Aquaculture Research Institute

Sample	Tag No.	Sex	Haplotype
AY1	136471470A	M	14
AY2	136471671A	F	10
AY3	136472255A	F	8
AY4	136472367A	F	6
AY5	136472621A	M	8
AY6	136472770A	F	10
AY7	136473163A	F	10
AY8	136474665A	F	10
AY9	136477256A	M	7
AY10	136477377A	F	10
AY11	136479456A	M	1
AY12	136709465A	M	2
AY13	136711365A	M	3
AY14	136718560A	M	1
AY15	136719191A	M	9
AY16	136719531A	M	1
AY17	136719793A	M	14
AY18	136721112A	F	9
AY19	136721766A	F	10
AY20	136722147A	F	2
AY21	136722217A	F	10
AY22	136722231A	M	10
AY23	136722486A	M	10
AY24	136723495A	M	9
AY25	136724611A	M	7
AY26	122644491A	F	7
AY27	115222583A	F	10
AY28	115251332A	M	2
AY29	115221457A	F	10
AY30	115239235A	F	10
AY31	122909121A	F	10
AY32	122924457A	F	10
AY33	114939654A	F	NA
AY34	122655555A	M	10
AY35	122655735A	M	14
AY36	122918697A	U	10
AY37	114933517A	M	10
AY38	115239552A	NA	10
AY39	114968213A	NA	9
AY40	122924537A	M	7
AY41	122713546A	F	7
AY42	122913371A	M	14
AY43	123212117A	M	7
AY44	122645650A	F	2
AY45	114632622A	F	10
AY46	114961614A	M	7
AY47	115316716A	F	2
AY48	115322767A	M	7
AY49	115258123A	F	3
AY50	115325595A	F	1
AY51	122911667A	M	1
AY52	122758214A	F	7
AY53	115311640A	F	2
AY54	115311514A	M	7
AY55	115228094A	F	2
AY56	115237232A	M	7

2. Chiangmai Inland Fisheries Research and Development Centre

Sample	Tag No.	Sex	Haplotype
CM1	137674673 A	M	NA
CM2	137917110 A	NA	2
CM3	137948114 A	F	1
CM4	140913562 A	M	2
CM5	137912674 A	M	2
CM6	140909163 A	M	2
CM7	137962254 A	NA	12
CM8	137926494 A	M	2
CM9	137973790 A	M	7
CM10	137967732 A	F	2
CM11	140909697 A	F	2
CM12	137939285 A	F	12
CM13	137926335 A	F	NA
CM14	140913464 A	M	2
CM15	137912366 A	F	2
CM16	137936621 A	M	2
CM17	137911622 A	NA	2
CM18	137909680 A	F	2
CM19	137979616 A	M	11
CM20	122746634 A	M	14
CM21	137909266 A	M	11
CM22	137909160 A	F	2
CM32	137926485 A	F	11
CM33	137677185 A	F	2
CM34	137956454 A	NA	2
CM35	137968185 A	NA	NA
CM36	137925161 A	F	2
CM37	137679477 A	F	2
CM38	137958392 A	NA	2
CM39	137967643 A	M	2
CM40	137927513 A	F	2

3. Kalasin Inland Fisheries Station

Sample	Tag No.	Sex	Haplotype
KS1	113719767A	NA	7
KS2	114563735A	NA	7
KS3	114565655A	NA	7
KS4	114611530A	NA	7
KS5	114624494A	NA	7
KS6	114934745A	NA	7
KS7	114951767A	NA	7
KS8	114973527A	NA	1
KS9	115224774A	NA	7
KS10	115229591A	NA	7
KS11	115233267A	NA	1
KS12	115237274A	NA	7
KS13	115254563A	NA	7
KS14	115255113A	NA	7
KS15	115261350A	NA	7
KS16	115311360A	NA	2
KS17	115311662A	NA	1
KS18	115314221A	F	10
KS19	115315314A	NA	10
KS20	115319092A	NA	2
KS21	115325224A	NA	1
KS22	115325295A	NA	5
KS23	122639370A	NA	7
KS24	122655526A	NA	1
KS25	122657124A	NA	1
KS26	122677373A	NA	7
KS27	122745290A	NA	7

4. Pitsanulok Inland Fisheries Research and Development Centre

Sample	Tag No.	Sex	Haplotype
PL1	122652183A	M	11
PL2	122655295A	F	4
PL3	155312250A	NA	11
PL4	114946386A	F	4
PL5	122913161A	NA	4
PL6	122209096A	M	4
PL7	114567257A	NA	4
PL8	122768122A	M	4
PL9	115312337A	F	4
PL10	115251266A	M	4
PL11	122644632A	M	4
PL12	115164627A	NA	1
PL13	123179266A	NA	4
PL14	122915324A	F	4
PL15	115251494A	NA	4
PL16	122712626A	NA	14
PL17	115226655A	M	4
PL18	122911225A	NA	4
PL19	122656240A	NA	12
PL20	115256573A	F	11
PL21	123179624A	M	4
PL22	122655091A	NA	4
PL23	122919465A	NA	4
PL24	122916761A	NA	4
PL25	155318111A	F	4
PL26	122915153A	F	4
PL27	122757240A	NA	7
PL28	122915173A	F	4
PL29	112916355A	NA	14

5. Taak Inland Fisheries Research and Development Centre

Sample	Tag No.	Sex	Haplotype
TK1	141427152A	NA	14
TK2	141475225A	NA	14
TK3	141711261A	NA	14
TK4	141445252A	NA	14
TK5	141461527A	NA	14
TK6	141712663A	NA	14
TK7	141709535A	NA	14
TK8	141444217A	NA	14
TK9	141277455A	NA	14
TK10	141469473A	NA	14
TK11	141479680A	NA	14
TK12	141435556A	NA	14
TK13	141469092A	NA	14
TK14	141469394A	NA	14
TK15	141445544A	NA	14

6. Songkhla Inland Fisheries Research and Development Centre

Sample	Tag No.	Sex	Haplotype
SK1	141467456A	NA	12
SK2	141479132A	NA	4
SK3	141711232A	NA	12
SK4	141453765A	NA	12
SK5	141448285A	NA	12
SK6	141451130A	NA	12
SK7	141436283A	NA	12
SK8	141474225A	NA	12
SK9	141717244A	NA	12
SK10	141467511A	NA	12
SK11	141719446A	NA	12

SK12	141713585A	NA	12
SK13	141472111A	NA	12
SK14	141476644A	NA	12
SK15	141458656A	NA	12
SK16	141476280A	NA	12
SK17	141464594A	NA	12
SK18	141472796A	NA	1
SK19	141472385A	NA	12
SK20	141713131A	NA	12
SK21	141466731A	NA	12
SK22	141711763A	NA	12

7. Phayao Inland Fisheries Research and Development Centre

Sample	Tag No.	Sex	Haplotype
PY1-2	140935573A	F	11
PY1-3	144568222A	F	2
PY1-4	144567094A	F	5
PY1-5	143317261A	F	2
PY1-6	143324243A	NA	1
PY1-7	144515240A	NA	2
PY1-9	144559792A	F	11
PY1-10	143673217A	NA	5
PY1-11	115239622A	F	2
PY1	122918772A	F	2
PY2	122918334A	F	11
PY3	115236793A	M	11
PY4	122767790A	F	11
PY5	143956726A	F	2
PY6	122746397A	M	2
PY7	115231321A	M	11
PY8	144975531A	M	11
PY9	144433160A	M	2
PY10	140939154A	F	2
PY11	122915191A	F	11
PY12	122919162A	F	11
PY13	122913797A	M	1
PY14	144767114A	M	1
PY15	144749246A	M	2
PY16	143311633A	M	2
PY17	144716315A	NA	7
PY18	123211694A	M	2
PY19	144723386A	M	12
PY20	144729185A	M	12
PY21	143312630A	M	2
PY22	115324743A	M	2
PY23	143929446A	M	14
PY24	144726092A	F	5
PY25	143676214A	M	2
PY26	122922622A	F	2
PY27	122923334A	M	2
PY28	122911352A	M	2
PY29	122913372A	F	11
PY30	122924550A	F	6
PY31	114975397A	F	2
PY32	143934330A	M	2
PY33	115226170A	F	2
PY34	114933555A	F	2
PY35	142469793A	M	2
PY36	143677797A	F	2
PY37	144767517A	M	2
PY38	113552321A	M	2
PY39	114912547A	M	1
PY40	143671097A	F	2
PY41	136814792A	M	2
PY42	144575390A	F	2
PY30-32-1	...574A	NA	
PY30-32-2	146147235A	NA	1
PY30-32-3	144731224A	NA	5
PY30-32-4	146164532A	NA	13
PY30-32-5	146129626A	NA	11
PY30-32-6	146222532A	NA	11
PY30-32-7	146173623A	NA	13
PY30-32-8	146279096A	NA	1
PY30-32-9	141436196A	NA	11
PY30-32-10	141456272A	NA	13
PY30-32-11	141446250A	NA	7
PY30-32-12	141454214A	NA	13
PY30-32-13	141463156A	NA	5
PY30-32-14	141454611A	NA	5
PY30-32-15	141479527A	NA	5
PY30-32-16	141477271A	NA	1
PY30-32-17	141477680A	NA	13
PY30-32-18	141451232A	NA	7
PY30-32-19	141479440A	NA	13
PY30-32-20	141449764A	NA	5
PY30-32-21	141477157A	NA	13
PY30-32-22	141462343A	NA	13
PY30-32-23	141459464A	NA	1
PY30-32-24	141454753A	NA	13
PY30-32-25	141461262A	NA	13
PY30-32-26	141471346A	NA	11
PY30-32-27	1141716790A	NA	13
PY30-32-28	141467392A	NA	11

Appendix 2. Allele frequencies and microsatellite diversity indices of MGC in 7 hatcheries

CM		loci						
	Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
	174					0.016		
	176					0.000		
	178					0.629		
	182					0.000		
	184					0.355		
	186					0.000		
	204			0.194				
	206			0.032				
	210			0.161				
	212			0.339				
	214			0.000				
	218			0.000				
	220			0.274				
	227	0.000						
	231	0.210						
	233						0.586	
	235	0.000						
	239	0.774					0.172	
	241						0.241	
	245	0.000						
	249	0.016						
	250				0.550			
	251							0.500
	252				0.200			
	253							0.016
	255							0.210
	256		0.032					
	257							0.242
	258		0.242		0.250			
	260		0.065					
	261							0.032
	264		0.145					
	270		0.129					
	274		0.387					
	Ho	0.387	0.871	0.774	0.900	0.613	0.690	0.903
	He	0.362	0.761	0.758	0.605	0.486	0.578	0.657
	A	3.000	6.000	5.000	3.000	3.000	3.000	5.000
	Ae	1.554	3.979	3.931	2.469	1.916	2.317	2.827
KS								
	Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
	174					0.000		
	176					0.000		
	178					0.463		
	182					0.056		
	184					0.481		
	186					0.000		
	204			0.093				
	206			0.000				
	210			0.519				
	212			0.056				
	214			0.111				

Appendix 2. (cont.)

KS							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
218			0.019				
220			0.204				
227	0.000						
231	0.259						
233						0.611	
235	0.000						
239	0.741					0.037	
241						0.352	
245	0.000						
249	0.000						
250				0.556			
251							0.259
252				0.407			
253							0.056
255							0.019
256		0.130					
257							0.648
258		0.333		0.037			
260		0.037					
261							0.019
264		0.000					
270		0.000					
274		0.500					
Ho	0.222	0.704	0.741	0.889	0.630	0.704	0.556
He	0.391	0.632	0.678	0.534	0.561	0.511	0.519
A	2.000	4.000	6.000	3.000	3.000	3.000	5.000
Ae	1.624	2.637	2.988	2.101	2.226	2.006	2.036
TK							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
174					0.000		
176					0.000		
178					0.800		
182					0.000		
184					0.200		
186					0.000		
204			0.000				
206			0.000				
210			0.033				
212			0.633				
214			0.000				
218			0.000				
220			0.333				
227	0.000						
231	0.233						
233						0.800	
235	0.000						
239	0.767					0.000	
241						0.200	
245	0.000						
249	0.000						
250				0.767			
251							0.267
252				0.000			

Appendix 2. (cont.)

TK							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
253							0.167
255							0.000
256		0.000					
257							0.567
258		0.300		0.233			
260		0.000					
261							0.000
264		0.300					
270		0.000					
274		0.400					
Ho	0.467	0.733	0.400	0.467	0.400	0.400	0.733
He	0.370	0.683	0.503	0.370	0.331	0.331	0.600
A	2.000	3.000	3.000	2.000	2.000	2.000	3.000
Ae	1.557	2.941	1.948	1.557	1.471	1.471	2.381
PY							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
174					0.000		
176					0.000		
178					0.608		
182					0.000		
184					0.392		
186					0.000		
204			0.184				
206			0.006				
210			0.259				
212			0.171				
214			0.051				
218			0.000				
220			0.329				
227	0.000						
231	0.346						
233						0.532	
235	0.000						
239	0.609					0.101	
241						0.367	
245	0.000						
249	0.045						
250				0.652			
251							0.361
252				0.152			
253							0.146
255							0.139
256		0.090					
257							0.291
258		0.205		0.196			
260		0.103					
261							0.063
264		0.167					
270		0.077					
274		0.359					

Appendix 2. (cont.)

	Ho	0.449	0.795	0.772	0.696	0.532	0.658	0.823
	He	0.511	0.782	0.764	0.517	0.480	0.576	0.745
	A	3.000	6.000	6.000	3.000	2.000	3.000	5.000
	Ae	2.030	4.480	4.147	2.055	1.912	2.338	3.854
PL								
	Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
	174					0.000		
	176					0.000		
	178					0.500		
	182					0.000		
	184					0.500		
	186					0.000		
	204			0.121				
	206			0.000				
	210			0.500				
	212			0.276				
	214			0.017				
	218			0.000				
	220			0.086				
	227	0.034						
	231	0.259						
	233						0.793	
	235	0.000						
	239	0.569					0.000	
	241						0.207	
	245	0.138						
	249	0.000						
	250				0.707			
	251							0.172
	252				0.069			
	253							0.448
	255							0.000
	256		0.446					
	257							0.379
	258		0.054		0.224			
	260		0.000					
	261							0.000
	264		0.000					
	270		0.000					
	274		0.500					
	Ho	0.586	0.500	0.793	0.483	0.448	0.414	0.586
	He	0.600	0.558	0.663	0.453	0.509	0.334	0.636
	A	4.000	3.000	5.000	3.000	2.000	2.000	3.000
	Ae	2.434	2.212	2.870	1.803	2.000	1.489	2.670
SK								
	Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
	174					0.250		
	176					0.023		
	178					0.500		
	182					0.000		
	184					0.227		
	186					0.000		
	204			0.023				
	206			0.205				
	210			0.045				

Appendix 2. (cont.)

SK							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
212			0.159				
214			0.000				
218			0.000				
220			0.568				
227	0.000						
231	0.432						
233						1.000	
235	0.000						
239	0.545					0.000	
241						0.000	
245	0.023						
249	0.000						
250				0.545			
251							0.250
252				0.000			
253							0.023
255							0.364
256		0.000					
257							0.341
258		0.182		0.455			
260		0.000					
261							0.023
264		0.000					
270		0.000					
274		0.818					
Ho	0.500	0.364	0.682	0.909	1.000	0.000	0.864
He	0.528	0.304	0.622	0.507	0.650	0.000	0.704
A	3.000	2.000	5.000	2.000	4.000	1.000	5.000
Ae	2.064	1.424	2.547	1.984	2.742	1.000	3.205
AY							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
174					0.000		
176					0.000		
178					0.518		
182					0.018		
184					0.446		
186					0.018		
204			0.080				
206			0.000				
210			0.455				
212			0.080				
214			0.107				
218			0.000				
220			0.277				
227	0.000						
231	0.339						
233						0.500	
235	0.018						
239	0.625					0.054	
241						0.446	
245	0.000						
249	0.018						
250				0.688			

Appendix 2. (cont.)

SK							
Allele	Pg1	Pg2	Pg3	Pg6	Pg16	Pg17	PSP507
251							0.384
252				0.232			
253							0.089
255							0.125
256		0.063					
257							0.357
258		0.393		0.080			
260		0.116					
261							0.045
264		0.152					
270		0.009					
274		0.268					
Ho	0.607	0.732	0.839	0.625	0.429	0.821	0.929
He	0.498	0.740	0.698	0.471	0.537	0.553	0.706
A	4.000	6.000	5.000	3.000	4.000	3.000	5.000
Ae	1.975	3.751	3.243	1.876	2.136	2.212	3.327

Appendix 3. Information on the six mating plans

1. Mating plan 1 (MP1, 24 pairs, male : female = 1:1)

Male	Female	Haplotype	r_{xy}
CM20	AY33	NA	-0.026
PY14	AY49	3	-0.019
PY8	CM12	12	-0.005
AY48	PY24	5	-0.003
CM5	CM3	1	0.006
CM16	PL2	4	0.007
PY38	AY41	7	0.017
AY14	PL14	4	0.017
PY39	AY44	2	0.018
PY13	PY1-3	2	0.020
AY15	PL28	4	0.021
PL17	AY20	2	0.022
PY27	PY1-2	11	0.024
PL8	CM22	2	0.024
AY5	CM36	2	0.025
PL11	PY36	2	0.031
AY17	CM10	2	0.033
AY42	PY29	11	0.035
AY37	AY53	2	0.040
PL6	PY1-4	5	0.042
AY16	PY2	11	0.048
PL21	AY18	9	0.050
PY19	AY32	10	0.051
PY37	CM32	11	0.051
		mean	0.022
		sd	0.021

2. Mating plan 2 (MP2; 48 pairs, male : female = 1:1)

Male	Female	Haplotype	rxy
CM20	AY33	NA	-0.026
PY14	AY49	3	-0.019
PY8	CM12	12	-0.005
AY48	PY24	5	-0.003
CM5	CM3	1	0.006
CM16	PL2	4	0.007
PY38	AY41	7	0.017
AY14	PL14	4	0.017
PY39	AY44	2	0.018
PY13	PY1-3	2	0.020
AY15	PL28	4	0.021
PL17	AY20	2	0.022
PY27	PY1-2	11	0.024
PL8	CM22	2	0.024
AY5	CM36	2	0.025
PL11	PY36	2	0.031
AY17	CM10	2	0.033
AY42	PY29	11	0.035
AY37	AY53	2	0.040
PL6	PY1-4	5	0.042
AY16	PY2	11	0.048
PL21	AY18	9	0.050
PY19	AY32	10	0.051
PY37	CM32	11	0.051
PY25	AY3	8	0.058
AY24	AY4	6	0.059
PY16	AY45	10	0.060
PY35	PY12	11	0.060
CM4	AY26	7	0.061
CM21	PY34	2	0.062
AY22	CM37	2	0.062
AY23	PY1	2	0.062
PY6	AY50	1	0.064
PY28	AY2	10	0.067
PY23	PY5	2	0.068
CM8	AY10	10	0.069
CM9	CM33	2	0.070
PY18	AY47	2	0.070
AY34	PY40	2	0.070
AY40	CM40	2	0.070
PY15	AY29	10	0.071
AY9	PY26	2	0.071
AY12	PL25	4	0.077
PY21	PL20	11	0.078
AY51	PY42	2	0.082
AY28	AY52	7	0.085
PY32	CM13	NA	0.086
AY43	PL4	4	0.092
		mean	0.046
		sd	0.029

3. Mating plan 3 (MP3; 69 pairs, male : female = 1:1)

Male	Female	Haplotype	r_{xy}
CM20	AY33	NA	-0.026
PY14	AY49	3	-0.019
PY8	CM12	12	-0.005
AY48	PY24	5	-0.003
CM5	CM3	1	0.006
CM16	PL2	4	0.007
PY38	AY41	7	0.017
AY14	PL14	4	0.017
PY39	AY44	2	0.018
PY13	PY1-3	2	0.020
AY15	PL28	4	0.021
PL17	AY20	2	0.022
PY27	PY1-2	11	0.024
PL8	CM22	2	0.024
AY5	CM36	2	0.025
PL11	PY36	2	0.031
AY17	CM10	2	0.033
AY42	PY29	11	0.035
AY37	AY53	2	0.040
PL6	PY1-4	5	0.042
AY16	PY2	11	0.048
PL21	AY18	9	0.050
PY19	AY32	10	0.051
PY37	CM32	11	0.051
PY25	AY3	8	0.058
AY24	AY4	6	0.059
PY16	AY45	10	0.060
PY35	PY12	11	0.060
CM4	AY26	7	0.061
CM21	PY34	2	0.062
AY22	CM37	2	0.062
AY23	PY1	2	0.062
PY6	AY50	1	0.064
PY28	AY2	10	0.067
PY23	PY5	2	0.068
CM8	AY10	10	0.069
CM9	CM33	2	0.070
PY18	AY47	2	0.070
AY34	PY40	2	0.070
AY40	CM40	2	0.070
PY15	AY29	10	0.071
AY9	PY26	2	0.071
AY12	PL25	4	0.077
PY21	PL20	11	0.078
AY51	PY42	2	0.082
AY28	AY52	7	0.085
PY32	CM13	NA	0.086
AY43	PL4	4	0.092
CM1	CM11	2	0.119
CM6	PL9	4	0.115
CM14	AY6	10	0.095
CM19	AY7	10	0.134
CM39	AY30	10	0.098
PY3	PY31	2	0.201
PY7	AY8	10	0.125
PY9	CM18	2	0.122
PY20	AY55	2	0.11
PY22	PL26	4	0.128
PY41	PY1-9	11	0.112
PL1	PY4	11	0.144
PL10	AY27	10	0.133
AY1	CM15	2	0.098
AY11	PY30	6	0.101
AY13	AY31	10	0.155
AY25	PY11		0.201
AY35	PY10	2	0.123
AY46	PY1-11	2	0.162
AY54	KS18		0.168
AY56	PY33	2	0.102
		Mean	0.072
			0.049

4. Mating plan 4 (MP4; 72 females with 31 males)

Female	Male	r_{xy}
AY33	CM20	-0.026
AY49	PY14	-0.019
CM12	PY8	-0.005
PY24	AY48	-0.003
PY1-3	CM20	0.001
CM3	CM5	0.006
PY1-4	AY48	0.007
PL2	CM16	0.007
AY53	PY14	0.013
PY34	CM20	0.016
PL14	AY14	0.017
AY41	PY38	0.017
AY44	PY39	0.018
CM36	PY14	0.020
PL28	AY15	0.021

AY20	PL17	0.022
CM22	PL8	0.024
PY1-2	PY27	0.024
AY18	PL8	0.024
PL4	PY39	0.025
PY36	PL11	0.031
CM10	AY17	0.033
PY29	AY42	0.035
PY5	PL8	0.036
AY26	PL8	0.036
CM32	PY27	0.038
PY40	PL8	0.038
PY26	AY5	0.041
CM13	PY8	0.043
PY2	PY14	0.043
PY4	AY48	0.044
AY3	AY42	0.048
PY42	PL17	0.049
PL20	PY14	0.049
AY52	AY14	0.049
AY27	PL8	0.050
CM18	PL21	0.051
AY32	PY19	0.051
PY1	PL8	0.054
PY12	PY27	0.054
PY30	PL17	0.056
PL25	PY38	0.056
AY50	PY27	0.056
AY4	AY24	0.059
AY45	PY16	0.060
CM37	AY22	0.062
PY11	PL8	0.065
PY1-9	AY42	0.066
PY33	PL11	0.066
AY2	PY28	0.067
AY31	CM5	0.067
AY55	PL8	0.068
AY10	CM8	0.069
AY29	PL6	0.069
CM33	CM9	0.070
CM40	CM9	0.070
PY31	CM8	0.070
AY30	CM8	0.070
AY47	PY18	0.070
CM15	AY16	0.072
PY10	PL11	0.073
PL26	AY15	0.074
KS18	AY48	0.076
PL9	PY38	0.077
AY21	PY6	0.078
AY8	CM5	0.081
AY6	PY37	0.082
AY7	PY37	0.082
PY1-11	PY28	0.084
CM11	CM21	0.089
PY1-5	CM5	0.090
AY19	CM8	0.093
	Mean	0.047
	sd	0.028

5. Mating plan 5 (MP5; all mtDNA haplotypes with same frequency, male:female = 1:1)

Female	Male	Haplotye	r_{xy}
AY49	PY14	3	-0.019
CM12	PY8	12	-0.005
PY1-3	CM20	2	0.001
CM3	CM5	1	0.006
PL2	PY32	4	0.007
PY1-4	AY48	5	0.007
PY24	AY40	5	0.011
PL14	AY14	4	0.017
AY41	PY38	7	0.017
PL28	AY15	4	0.021
AY20	PL17	2	0.022
AY18	PL8	9	0.024
PY36	PL11	2	0.031
PY1-2	AY5	11	0.041
AY3	AY42	8	0.048
PY2	AY16	11	0.048
AY32	PY19	10	0.051
AY50	PY27	1	0.056
AY4	AY24	6	0.059
AY26	PL21	7	0.059
PY29	AY12	11	0.059
AY45	PY16	10	0.06
PY30	PY39	6	0.065
AY52	PY23	7	0.074
		mean	0.032
		SD	0.026

6. Mating plan 6 (MP6;)

Male	Female	Haplotype	r_{xy}
PY14	AY49	3	-0.019
PY8	CM12	12	-0.005
AY48	PY24	5	-0.003
CM20	PY1-3	2	0.001
CM5	CM3	1	0.006
CM16	PL2	4	0.007
AY14	PL14	4	0.017
PY38	AY41	7	0.017
PY39	AY44	2	0.018
AY15	PL28	4	0.021
PL8	AY18	9	0.024
PY27	PY1-2	11	0.024
AY5	CM36	2	0.025
PL11	PY36	2	0.031
AY17	CM10	2	0.033
PL21	AY20	2	0.034
AY37	AY53	2	0.040
PL6	PY1-4	5	0.042
AY42	AY3	8	0.048
AY16	PY2	11	0.048
PY19	AY32	10	0.051
PY37	CM32	11	0.051
PL17	PY30	6	0.056
CM21	PY34	2	0.062
		mean	0.026
		SD	0.0213