HOW TO KEEP NUMBERS UP AND INBREEDING DOWN IN SUPPLEMENTED STOCKS

Fiumera, A. C., B. A. Porter, G. Looney, M. A. Asmussen and J. C. Avise. 2004. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. *Conservation Biology* 18:94-101.

Abstract: Supplemental breeding is an intensive population management strategy wherein adults are captured from nature and spawned in controlled settings, and the resulting offspring are later released into the wild. To be effective, supplemental breeding programs require crossing strategies that maximize offspring production while maintaining genetic diversity within each supplemental year class. We used computer simulations to assess the efficacy of different mating designs to jointly maximize offspring production and maintain high levels of genetic diversity (as measured by the effective population size) under a variety of biological conditions particularly relevant to species with high fecundity and external fertilization, such as many fishes. We investigated four basic supplemental breeding designs involving either monogamous pairings or complete factorial designs (in which every female is mated to every male and vice versa), each with or without the added stipulation that all breeders contribute equally to the total reproductive output. In general, complete factorial designs that did not equalize parental contributions came closest to the goal of maximizing offspring production while still maintaining relatively large effective population sizes. Next, we estimated the effective population size of 10 different supplemental year classes within the breeding program of the robust redhorse (*Moxostoma robustum*). Two year classes failed to produce progeny, whereas successful year classes used partial factorial designs to realize effective sizes ranging from 2 to 26 individuals. On average, a complete factorial design could increase the effective size of each robust redhorse supplemental year class by 19%.

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DETECTING DISEASE-MARKER ASSOCIATIONS IN SHRIMP

Hizer, S. E., T. M. Wright and D. K. Garcia 2004. Genetic markers applied in regression tree prediction models*. Animal Genetics* 35:50-52.

Abstract: Classification and regression tree (CART) modelling was used to determine infectious hypodermal and haematopoietic necrosis virus (IHHNV) resistance and susceptibility in *Penaeus stylirostris*. In a previous study, eight random amplified polymorphic DNA (RAPD) markers and viral load values using real-time quantitative PCR were obtained and used as the training data set in order to create numerous regression tree models. Specifically, the genetic markers were used as categorical predictor variables and viral load values as the dependent response variable. To determine which model has the highest predictive accuracy for future samples, RAPD fingerprint data was generated from new *Penaues stylirostris* IHHNV resistant and susceptible individuals and used to test the regression models. The best performing tree was a four terminal node tree with three genetic markers as significant variables. Marker-assisted breeding practices may benefit from the creation of regression tree models that apply genetic markers as predictive factors. To our knowledge this is the first study to use RAPD markers as predictors within a CART prediction model to determine viral susceptibility.

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USEFUL DIVERSITY INFORMATION ON CULTURED TILAPIA

Romana-Eguia, M. R., M. Ikeda, Z. U. Basiao (2004). Genetic diversity in farmed Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA analysis. *Aquaculture* 236:131-150.

Abstract: We analyzed microsatellite and mitochondrial DNA restriction fragment length polymorphism (mtDNA-RFLP) in two domesticated (NIFI and Israel) and four genetically improved (GIFT, GMT, FAC-selected and SEAFDEC-selected) Nile tilapia (*Oreochromis niloticus*) as well as five red hybrid tilapia (*Oreochromis mossambicus x_O. niloticus*) stocks (BFS, FACred, NIFIred, HL, and PF) farmed in Asia. Microsatellite variation at five loci (UNH216, UNH172, UNH123, UNH147,UNH222) was more informative in characterizing stock differences than the mtDNA-RFLP markers that were based only on 14 restriction morphs. Contemporary microsatellite data showed that GIFT Nile tilapia had the highest mean expected heterozygosity (He=0.813), while GMT had the lowest (He=0.666). The unselected NIFI stock and SEAFDEC-selected were genetically similar, while GMT differed significantly from the other Nile tilapia stocks. Among the red tilapias, NIFIred had the highest He (0.715), while BFS had the lowest variability (He=0.567). The Taiwanese red tilapia HL and Thai NIFIred were genetically similar. Except for NIFI, most of the Nile and red tilapia stocks exhibited remarkably significant homozygote excess relative to Hardy–Weinberg Equilibrium (HWE), suggesting some degree of inbreeding. Asian Nile tilapias were more genetically diverse (pooled He=0.791; mtDNA nucleotide divergence value dA=0.009) than the red tilapias (pooled He=0.697; mean dA=0.004). This slight divergence between the Nile and red tilapias was also seen in the analysis of molecular variance (AMOVA; FCT=0.0018) and in genetic distance and nucleotide divergence dendrograms. However, the AMOVA revealed that the greater percentage of variation (99.33%) in the total genetic diversity of the surveyed stocks is principally due to differences at the individual level and not between nor

within groups. The significance of these results is that they reflect and lead to new inferences regarding the selective breeding and culture methods used in managing these farmed stocks.

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A BREEDING PLAN THAT MINIMIZES INBREEDING

Fernández, J., M. A. Toro and A. Caballero. 2003. Fixed contributions designs vs. minimization of global coancestry to control inbreeding in small populations. *Genetics* 165:885-894.

Abstract: Populations with small census sizes are at risk because of the loss of genetic variability and the increase of inbreeding and its harmful consequences. For situations with different numbers of males and females, several hierarchical designs have been proposed to control inbreeding through the fixation of individuals' contributions. An alternative method, based on the minimization of global coancestry, has been proposed to determine contributions as to yield of the lowest levels of inbreeding in the population. We use computer simulations to assess the relative efficiency of the different methods. The results show that minimizing the global coancestry leads to equal or lower levels of inbreeding in the short and medium term, although one of the hierarchical designs provides lower asymptotic inbreeding rates and, thus, less net inbreeding in the long term. We also investigate the performance of the alternative methods against departures from the ideal conditions, such as inbred or differentially related base individuals and random failures in the expected contributions. The method of minimization of global coancestry turns out to be more flexible and robust under these realistic situations.

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USEFUL SHRIMP (*P. MONODON*) MICROSATELLITES

Wuthisuthimethavee, S., P. Lumubol, a. Vanavichit and S. Tragoonrung. 2003. Development of microsatellite markers in black tiger shrimp (*Penaeus monodon* Fabricius). *Aquaculture* 224:39-50.

Abstract: Microsatellites or Simple Sequence Repeats (SSRs) represent an abundant source for genetic markers in eukaryotic genomes. Nine synthesized repeat sequences labeled with biotin were used to create an enriched microsatellite library [(AG)10, (TG)10, (GAA)10, (GAC)10, (CAT)10, (TAC)10, (GACA)8, (GATA)8, and (TCAG)8] for *Penaeus monodon*. From a total of 2417 clones, only 406 clones (16.8%) were positive after colony hybridization against the nine repeat sequences. Those clones with insert sizes ranging from 300 to 1000 bp were isolated and characterized. The most abundant repeat sequences in the *P. monodon* genome are (AG)n and (CAT)n making up to 22% and 21%, respectively. A total of 102 from the 129 primer pairs (designed for 129 clones chosen) were able to amplify *P. monodon* DNA. According to the sequences of the 102 clones, 27 loci, 17 loci, 4 loci, and 54 loci were dinucleotide, trinucleotide, tetranucleotide, and compound repeat sequences, respectively. Thirty microsatellite primer pairs were used to screen wild *P. monodon* germplasm and the result revealed PIC values ranged from 0.4275 to 0.9264. Therefore, this set of microsatellite primers would provide a useful tool in P. monodon breeding programs.

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BREEDING SCHEME THAT MAXIMIZES ALLELE DIVERSITY

Vales-Alonso, J., J. Fernández, F. J. González-Castaño and A. Caballero. 2003. *A parallel optimization approach for controlling allele diversity in conservation schemes. Mathematical Biosciences* 183:161-173.

Abstract: We propose a novel method to control allelic diversity in conservation schemes based on an optimization problem, characterized by a convex program subject to integer linear constraints. Departing from previous studies considering similar problems, we implement a parallel simulated annealing algorithm to minimize the number of alleles lost across generations. The proposed algorithm shows excellent timing and minimization performances. Execution time decreases linearly with the number of processors used, providing similar results in all cases.

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INTROGRESSION IN CATFISH IN THAILAND

Uthairat Na-Nakorn, Wongpathom Kamonrat, and Thawatchai Ngamsiri (2004). Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. *Aquaculture* 240: 145-163

Abstract: The Thai walking catfish, *Clarias macrocephalus* Günther, 1864, is economically important to Thailand. It occupies marshes and swamps that are severely endangered due to population expansion and natural populations are thought to be suffering from massive back-crossing with the *C. macrocephalus*×*C. gariepinus* hybrids. Therefore, a study on genetic diversity of this species is required to enable efficient conservation and management plans. In this study, 25 natural populations were collected throughout the country, 12 populations from provinces locate in the Chaophraya river basin in the center of the country, 5 from the Mekong river basin, 1 from the east and 7 from the south. One population of hatchery origin was obtained from the Department of Aquaculture, Kasetsart University in Bangkok.

Twelve isozymes and one protein system were analyzed. Among 18 loci resolved, 8 were polymorphic. The number of alleles per locus, average polymorphism and individual polymorphism were significantly higher in collections from the Chaophraya river basin than from the Mekong, east and south. The hatchery population also had relative high genetic variation. Six out of twenty-six populations differed significantly from Hardy-Weinberg equilibrium after Bonferroni correction. None of loci pairs showed significant linkage disequilibrium after Bonferroni correction. The F_{st} value across loci was highly significant from zero. A neighbor-joining tree reveals that populations from the south were genetically distinct from the remaining populations.

Alleles peculiar to the African catfish [*C. gariepinus* (Burchell, 1822)] genome were observed in 12 of the natural populations and the hatchery population. This is evidence of genetic introgression which has probably persisted for several generations, since there was no significant genotype disequilibrium between the *macrocephalus* and *gariepinus* alleles at three diagnostic loci.

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GENETIC STUDIES OF ASIAN AROWANA

Tang PY[1], Sivanantahn J, Pillay SO, Muniandy S (2004). Genetci structure and biogeography of Asian arowana (*Scleropages formosus*) determinted by microsatellite and mitochondrial DNA analysis. *Asian Fisheries Science* 17: 81-92

Abstract: The Asian arowana (*Scleropages formosus*) is distributed in Southeast Asia and highly endemic to many river systems. Genetic structure of five strains of arowana was assessed. Twenty-nine microsatellite loci were screened to assess the sort-term genetic differentiation. Sequences of ATPase subunit 6 and 8 were obtained to estimate the time of divergence. Microsatellite data yielded high value of F_{ST} between strains. The gene tree constructed based on microsatellite data shows that the Asian arowana is a monophyletic group with two linages. The green arowana is the out group and has a closer relationship with Indonesian gold arowana. Sequences of the ATPase gene of the arowana were not as variable copared with microsatellites. The mtDNA yielded a gene tree of different topology as compared to that obtained from microsatellites. The arowana consists of a monophyletic group of mtDNA with three different lineages which represent three different colour: red, green and gold. The divergence of the different colour strains of arowana was backdated to between the late Pliocene to late Pleistocene era. It is believed that the arowana dispered in South East Asia when Sundaland was formed. The fluctuation of sea level during Pleistocene separated the Indoneasian islands with the Southeast Asian mainland and caused the arowana to diverge into distinct strains.

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