Genomic Evaluation of Haddock (*Melanogrammus aeglefinus* L.) broodstock

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Genomic evaluation of haddock (*Melanogrammus aeglefinus L.*) broodstock

by

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ABSTRACT

Martin-Robichaud, D.J., Haché, R. Pernet, F., and Ritchie, R. 2006. Genomic evaluation of haddock (*Melanogrammus aeglefinus* L.) broodstock. Can. Tech. Rep. Fish. Aquat. Sci. 2704: iv + 10 pp.

To preserve all previous efforts made in the development of haddock aquaculture in the Canadian Maritime provinces it was essential to support the continued maintenance and improvement of the existing haddock broodstock as a foundation for future aquaculture diversification. Industry and government representatives joined resources to secure the existence and genetic improvement of haddock broodstock for future commercial marine finfish culture. Various year-classes of F1 and F2 cultured haddock were selected based on their growth performance and maintained at two research facilities. Genotyping was done using five DNA microsatellite markers and the genomic data was consolidated into a common database. Genotype data from both groups of fish were compared and showed a high level of relatedness within sites. In Shippagan, 156 (of 218) fish were related to at least one other at the sibling level and all but one was related to at least one other at the half-sibling level. In comparison, 165 (of 248) fish from the St. Andrews Biological Station SABS were related to at least one other at the sibling level while 220 (or 248) were related to at least one other at the half-sibling level. Results indicate that although some fish at both sites originate from the same parental stock the two populations are now genetically quite distinct. Even with high relatedness among fish, it is possible to improve the genetic heterozygosity of both stocks through planned breeding. To maximize the effective breeding stock, fish should be shared between the two sites since fish at each site contain unique alleles and additional wild stock should be incorporated into further breeding endeavors.

RÉSUMÉ

Martin-Robichaud, D.J., Haché, R. Pernet, F., and Ritchie, R. 2006. Genomic evaluation of haddock (*Melanogrammus aeglefinus* L.) broodstock. Can. Tech. Rep. Fish. Aquat. Sci. 2704 : iv + 10 pp.

La préservation des efforts antérieurs placés dans le développement de l'aquaculture de l'aiglefin dans les Provinces maritimes du Canada nécessite un appui continu pour le maintien et l'amélioration des géniteurs d'aiglefins existants. Ces géniteurs d'aiglefins représentent la base pour la diversification future de l'industrie de l'aquaculture. Les représentants de l'industrie et des gouvernements ont mis en commun leurs ressources afin de sécuriser les géniteurs d'aiglefins existant et d'améliorer les connaissances génétiques de ces spécimens en vue d'une commercialisation future de cette espèce marine. Les aiglefins adultes de diverse classes de F1 et F2 ont été sélectionnés en se basant sur leur taux de croissance puis ont été transférés aux deux Centres de recherche. Le génotype a été déterminé en utilisant cinq différents marqueurs d'ADN du type microsatellite et les données génomiques ont été consolidées dans une base de données commune. Les données génomiques des deux groupes de géniteurs ont été par la suite comparées. Cette comparaison a démontré un taux de consanguinité élevé pour chacun des sites. À Shippagan, 156 poissons sont apparentés au niveau de la fratrie à au moins un autre et

tous sauf un sont apparentés au niveau demi-frère. Au SABS, 165 poissons sont apparentés au niveau de la fratrie à au moins un autre alors que 220 sont apparentés au niveau demi-frère. Les résultats obtenus indiquent que les poissons des deux sites représentent maintenant deux populations distinctes même s'ils du même stock parental. Malgré un degré de parenté élevé entre les poissons, il est toujours possible d'améliorer l'hétérozygocité des deux populations en planifiant adéquatement les stratégies de reproduction. Les poissons devraient être échangés entre les deux sites afin de maximiser les stratégies de reproduction puisqu'il y a des poissons possédant des allèles uniques à chacun des sites. L'incorporation de géniteurs sauvages devrait être envisagée afin d'optimiser les efforts futur de croisements.

INTRODUCTION

A goal of the Canadian aquaculture industry is to diversify and develop a commercially and environmentally sustainable aquaculture industry producing prime, white flesh fish for market. The development period for a new farmed product is typically very long, with numerous technical challenges, and is usually a very high cost undertaking. Significant public and private investments and time have gone into getting marine finfish aquaculture to the stage it is today (Fig. 1). For Canadian haddock culture to make significant production gains, to prove the potential of haddock culture, and to develop a competitive edge internationally, research on genetic selection has to begin at once as the process and returns are long-term. The phenotypic response of fish to genetic selection for improved growth is considered higher than that of terrestrial animals. The genetic variance (CV) in fish ranges from 20-35% compared to a CV of 7-10% in terrestrial farm animals (Gjedrem 1998). Therein lies the opportunity and the challenge to conduct selection in a sustainable process.

Due to the short generation time and high fecundity, significant growth gains can be achieved with a relatively low number of finfish broodstock; however, it is inevitable that inbreeding and loss of genetic variability (as apparent by fewer alleles at loci) will eventually affect performance and impede further genetic improvements. It is crucial to establish a domesticated stock with a large and genetically diverse initial breeding stock with established family structure. Breeding programs intended for sustainable aquaculture should also include ecological and social elements in genetic selection by incorporating non-market and market values into their complex and long-term programs (Olesen et al. 2003).

A discussion of population genetics, particularly that associated with commercial broodstock, should begin with a clear understanding of the two types of populations: evolving populations and those in equilibrium (non-evolving). While there is not necessarily a universally optimum state for a population, population studies often begin with a comparison to populations in a non-evolving state, more commonly called Hardy-Weinberg Equilibrium (HWE). Populations in HWE are usually: (i) large, (ii) genetically isolated, (iii) have negligible mutations, (iv) have randomly mating individuals, and (v) no natural selection. Given these assumptions, a population's genotype and allele frequencies will remain unchanged over successive generations, and the population is said to be in HWE equilibrium. Comparison of a population to one in HWE enables us to compare a population's actual genetic structure over time with the genetic structure we would expect if the population were in HWE equilibrium (i.e., not evolving). If genotype frequencies differ from those we would expect under equilibrium, we can assume that one or more of the HWE assumptions is being violated, and attempt to determine which one(s). This is a key to unlocking differences between populations.

Previous attempts at commercialization of haddock culture relied initially on a small number of wild broodstock. This is contrary to well known requirements for a large and diverse effective breeding stock (N_e). Selection of F_1 broodstock began with little or no genetic information pertaining to family structure. This exposes the industry to a great risk of inbreeding and loss of significant levels of genetic variation through the course of domestication. It is our goal to use biotechnology and genetic selection protocols to: 1) maintain and improve the existing broodstock at two institutions; 2) direct communal spawning

with unrelated fish; and 3) consolidate genetic and morphological data from all haddock holding sites in the Maritimes. These efforts will allow the establishment of a comprehensive haddock broodstock genetic database. With this foundation firmly established, the culture of haddock in Canada may be sustained until economic conditions and opportunities for industry expansion and commercialization are realized.

The Coastal Zones Research Institute in Shippagan, New Brunswick (CZRI) and the Fisheries and Oceans Biological Station in St. Andrews, New Brunswick (SABS) are the primary research institutions ensuring the existence of selected broodstock. The broodstock at both facilities have developed over the last 10 yr from wild stock to various F1 and F2 generations with little directed breeding as fish spawned in communal tanks. These fish were genotyped using DNA microsatellite markers and various parameters of genetic relatedness evaluated. CZRI also continued the production of haddock juveniles for performance trials through the support of the New Brunswick Department of Agriculture and Aquaculture (NB DAA) and Cooke Aquaculture Inc.

PROJECT OBJECTIVES

The objectives of this study were to selectively genotype and sex haddock retained as broodstock at CZRI and SABS. The intention was to consolidate genetic and morphological data pertaining to all haddock broodstock maintained at both sites into one database. Having stock split between two sites was a necessary security measure. Based on the genetic composition of each stock, fish will be reallocated to production sites as necessary to maximize the genetic heterozygosity and to minimize inbreeding risk. This analysis would also indicate the need for new stock. This is the basic evaluation necessary until further interest and resources are available for a more thorough molecular and quantitative genetic breeding program.

METHODS

BROODSTOCK HISTORY

Mature wild haddock were collected in the Bay of Fundy in 1996, 1997 and 1998 and distributed to both CZRI and SABS. Eggs from these fish supplied the juvenile production requirements of three research hatcheries in the Canadian Maritime provinces from 1997 to 2001. Eggs were transferred between these institutions to maximize the reproductive capacity of broodstock used for production trials. Juveniles were selected during each production cycle to establish a domesticated broodstock with preferred characteristics primarily attributable to their life history during culture, specifically that they survived culture conditions. The haddock broodstock progressed from wild stock to selected F1 and F2 fish over the last 10 yr. Communal spawning was used for all production; therefore, pedigree details were not available. Results from this study allowed the application of DNA microsatellite genotyping to evaluate the relatedness of current broodstock and improve the allocation of fish at hatcheries based on genotype and sex.

FIN CLIP AND SEX RATIO DETERMINATION

The sex of haddock broodstock at both sites was determined using ultrasound. Fin clips were collected and stored in 70% ethyl alcohol for genotyping. Ultrasonography allows gender determination in a non-lethal manner so a suitable sex-ratio of fish could be maintained in communal spawning tanks (Martin-Robichaud and Rommens 2001) (Fig. 2). The ultrasound used in this project was a Sonosite Titan portable Ultrasound with an L-38 linear Transducer (5-10 Mhz).

GENETICS ANALYSIS

Multiplex PCR

DNA was extracted from fin clips using the Wizard SV 96 (Promega) extraction kit according to the manufacturer's instructions. One µl of DNA was used in each 15 µl PCR reaction. Each sample was amplified using two PCR reactions. The first PCR reaction included fluorescently labeled primers Mae13-FAM (0.25 µM), Mae7-NED (0.12 µM) and GMO34-HEX (0.75 µM) and the second reaction included Mae9-FAM (0.25 µM) and Mae46-NED (0.2 µM) (Table 1) (Miller et al. 2000; O'Reilly et al. 2002). The PCR reaction mixture included 2 mM $MgCl_2$ (Applied Biosystems), 1X PCR Buffer II (Applied Biosystems), 0.1 mM dNTP (Invitrogen), and 1 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems). The PCR amplification conditions consisted of 94°C for 11 min, 35 cycles of 94°C for 15 s, 52°C for 20 s, 72°C for 45 s and followed by 72°C for 60 min. All amplifications were done using a GeneAmp 9600 or a GeneAmp 9700 (Applied Biosystems).

For genotyping, 1.0 µl of the PCR reagent was combined with 12.0 µl of HI/DI Formimide (Applied Biosystems), and 0.5 µl of 400HD ROX (Applied Biosystems), the internal size standard. The samples were then heated at 95°C for 5 min followed immediately by 5 min on ice. The samples were then loaded into an ABI3100 genetic analyzer (Applied Biosystems) and the data was analyzed using Genotyper v3.7 or Genemapper v3.5 software (Applied Biosystems). Genotype, sex, size and year-class data was compiled for the 1999, 2000, 2001, and 2003 year-classes.

Database Construction

Information relating to haddock broodstock was compiled from existing CZRI and SABS records. Records contained information on year-class, PIT tag number, sex, size, genotype relatedness (as indicated by the number of half and full sibs to which it is related), tank location and mortality. However, not all information was available for all fish.

Relatedness Analysis

Genetic data from current stocks were formatted and analyzed using the Excel Microsatellites macro software (Stephen Park, Department of Genetics, Trinity College, Dublin 2, Ireland). This macro details allele frequencies and heterozygosity for a particular locus. Data

in Genepop format[1] were then analyzed with the Genepop program (http://wbiomed.curtin.edu.au/genepop/genepop_op1.html) to determine genic and genetic variance, variance from Hardy-Weinberg equilibrium, and general relatedness of animals in a population. Unless otherwise specified, the following parameters were used: Dememorization number 1000, Number of batches 100, Number of iterations per batch 1000. A more detailed relatedness analysis, detailing the relatedness between each animal to each other in a particular population, was performed using Momentary Estimate of Relatedness (MER) freeware (Wang 2002). This program was run using default parameters. The total relatedness estimates for each pair of fish (Mean(R)) was determined. A Mean(R) of 1.0 indicates complete sharing of genetic material (identical fish). A Mean(R) of 0.5 or greater indicates sharing of half the genetic material (e.g. parent-offspring or sibling-sibling). Similarly a score of 0.25 indicates the two fish share a quarter of the genetic material (e.g. half-siblings).

RESULTS

POPULATION STUDY

To initiate a preliminary breeding plan, haddock broodstock were genotyped so as to maximize genetic diversity in future generations until a more thorough breeding plan to evaluate trait characteristics and family variability could be done. Genotype data corresponding to stocks held at SABS (209 fish from 2003 year-class) and Shippagan (218 from 2003 and 2000 year-classes) were compared using basic population statistics. The number of alleles and unique alleles were determined for each population (Table 2). The CZRI population possessed a larger number of alleles and had a larger number of unique alleles suggesting that the CZRI population was more genetically diverse (heterozygous) than the SABS population. Figures 3A-E shows the allele distribution and frequency for each population.

Relatedness Analysis

The genetic data for each population was analyzed with Genepop to test the H_0: "*There is random union of gametes (population equilibrium)*". The probabilities for this were extremely small and only the Mae7 locus in fish at CZRI was found to be in equilibrium (P-val = 0.0211, S.E. = 0.0043). However, this may be explained by the fact that there were only seven alleles detected at this locus. All other loci were not in equilibrium. This is not surprising given the stock size, spawning behavior and communal spawning dynamics of this species.

Because the populations were not in Hardy Weinberg Equilibrium, it was not possible to compare the genotype distribution at each locus between the two populations. Instead, the allelic distribution at each locus of the two populations was compared using Genpop (Ho: "*The allelic distribution is identical across populations*"). However, the two populations did not have similar allelic distributions at any locus (p value < 0.05).

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[1] Data is converted into a 3-digit genepop format. In this format, the population name is followed by the allele data, presented as 6-digit numbers (3-digit alleles at the same locus are joined together e.g. 302306 - alleles smaller than 100 bp are prefixed with zeros). Missing data is given as zeros (000000). The heading 'POP' is inserted between each population.

When the different loci were tested for independent assortment (H_0: "*Genotypes at one locus are independent from genotypes at the other locus*" (Table 3), all of the loci pairs in the SABS population and the majority of pairs in the CZRI stock were shown to reject the null hypothesis, indicating that they were not assorting independently. This is in part a reflection of the population disequilibrium and indicates a bottleneck in the each population. The effect is greatest in the SABS population.

A good indication of the relatedness and genetic diversity of a population is the number of individuals within a population which are identical (or nearly identical). In the SABS population, a total of 20 pairs of fish (out of a population of 209) were identified as having the same genotype at all five loci. By contrast only four pairs of fish with identical genotypes were found in the CZRI population (218 fish) and no fish in the CZRI population was identical to any in the SABS population.

Following this initial relatedness analysis, the two populations were analyzed with MER software to determine a relatedness value for each fish in each population. This would allow identification of highly related fish which could be removed from future spawnings. In this analysis the genotype of each fish is compared to each other fish in the database, and assigned a score based on its relatedness to *each* other fish. A score of 1.0 indicates 100% sharing of the genotype, a score of 0.5 indicates a 50% sharing in genotype (full sib) and a score of 0.25 indicates a 25% sharing of genotype (half sib). For reasons of computational size it was not possible to analyze more than about 250 fish at once so we ran separate analyses for CZRI and SABS stock. For CZRI stock we were able to run all broodstock studied in the previous section (218 fish) through MER, and for the SABS fish we studied all living broodstock from the 1999, 2000 and 2003 year-class (248 fish) through the database. Of the 218 haddock at CZRI, 156 showed full sib relatedness to at least one other fish at the full-sib level, and all but one (217 fish) were related to at least one other fish at the half-sib level. Twelve fish of these fish were each related to at least 10 other fish at the full sib-level, and a total of 415 others at the half-sib level revealing the closely related nature of fish in the CZRI population. The male: female ratio for the highest and lowest relatedness groups was approximately equal indicating that the both males and females showed equally high relatedness.

In SABS broodstock 165 of 248 fish were related to at least one other fish at the full sib level and 220 were related to at least one other at the half-sib level. Overall 95 fish were related to at least 10 other fish at the sib-level. This is in great contrast to the 12 fish in the CZRI population where each related to at least 10 fish at the sibling level. This underscores the relatedness of the population as shown in the allele frequencies. However the multi-year-class analysis of the SABS population revealed very little relatedness between year-classes (e.g. 1999 and 2003 or 2000 and 2003) likely underscoring the separation of these year-classes at spawning and the variation in spawning between fish in each spawning group.

DISCUSSION

Analysis of current CZRI and SABS haddock populations, showed reduced heterozygosity and different allele frequencies and genotypes. This suggests that each has evolved from their founding population (wild stock collected in 1996, 1997 and 1998) and neither population is in

HWE. However, *together* the two populations contain considerable genetic diversity, and considerable genetic potential. Although wild parental genotypes were not available for comparison we can assume that genetic diversity within each population has been reduced due to loss of alleles and genetic drift. This has been documented for other newly commercialized marine finfish originating from a small stock of wild parents (Jackson et al. 2003). Disequilibrium may be due to a relatively small population size and is probably exacerbated by communal spawning where it is difficult to control the number and frequency of fish contributing to offspring. In gadoid culture with communal spawning, dominant males may sire a disproportionate number of offspring thereby reducing N_e further. It is interesting to note that of the remaining 28 fish from the SABS population with no half-sib pairs in the population, 19 were from the 2000 year-class and 9 were from the 2003 year-class. This suggests that in the SABS population, little inbreeding occurred between year-classes, or that some fish did not contribute to the spawning populations. This represents an opportunity to increase the genetic diversity for future generations through the establishment of specific mating pairs or pools. Nevertheless, it is possible to improve the genetic diversity of both stocks through carefully planned spawning. For maximum benefit and efficiency, fish should be shared between the two facilities; to distribute unique alleles not shared and specific matings should be established. A relatedness analysis has been performed on the CZRI and SABS populations, and a list of the most highly related fish has been developed and can be used to refine the breeding stocks. The data will need to be reanalyzed and evaluated depending on when a breeding program is started and the extent of mortalities up to that point. At this early stage of development most genetic improvements made to this stock arise from domestication pressures. Therefore the imperative addition of wild stock would do little to diminish any specific selective advantages already attained. If the spawning population is kept large and inbreeding low by using many breeding fish per generation then selection can continue for many generations without severe loss of genetic gain (Oelsen et al., 2003).

The high number of females in the CZRI stock is probably due to the selection of large fish from the cages. Growth data and personal observations have shown that females are usually larger than males. This indicates that the establishment of all-female stocks may be advantageous to industry. To advance haddock culture, breeding programs should be conducted to ensure sufficient genetic diversity is available for selection of beneficial commercial traits.

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Table 1: Haddock primers used for genotyping with multiplex PCR.

Multiplex	Marker Name	Primer Sequence (5'-3')	Dye
A	Mae7	F: CACACCGAGATAAACCAA R: AAAGCCTCTTGTACTAACTG	Ned
B	Mae9	F: GGATGCCCAGCTTAGGAT R: TGGAACTGGGTAGTGTAAC	Fam
A	Mae13	F: GCTATTGGGTTGGAACA R: GCAGGCGTTTCAATTAGA	Fam
B	Mae46	F: AAATAATGCCGCTATCAG R: TATAACAACCAAACCAAACAA	Ned
A	Gmo34	F: GGTTGGACCTCATGGTGAA R: TCCACAGAAGGTCTCCTAA	Hex

Table 2: Number of alleles at each loci in each population.

Locus	CZRI	SABS
Mae7	6(1)	5
Mae9	36 (12)	24
Mae13	44 (19)	25 (3)
Mae46	16 (2)	14
Gmo34	26 (9)	20 (3)

() denotes number of unique alleles.

Table 3: Test for independent assortment of pairs of loci within each population.

Locus 1	Locus 2	CZRI		SABS	
		P-value	S.E.	P-value	S.E.
Mae7	Mae9	0.00000	0.00000	0.00000	0.00000
Mae7	Mae13	0.00000	0.00000	0.00000	0.00000
Mae9	Mae13	0.03558	0.01794	0.00000	0.00000
Mae7	Mae46	0.00000	0.00000	0.00000	0.00000
Mae9	Mae46	0.00801	0.00801	0.00000	0.00000
Mae13	Mae46	0.09142	0.02875	0.00000	0.00000
Mae7	Gmo34	0.00822	0.00822	0.00000	0.00000
Mae9	Gmo34	0.05898	0.02348	0.00484	0.00484
Mae13	Gmo34	0.19812	0.03987	0.00000	0.00000
Mae46	Gmo34	0.32309	0.04677	0.00000	0.00000

Fig. 1. Haddock juveniles in sea cages.

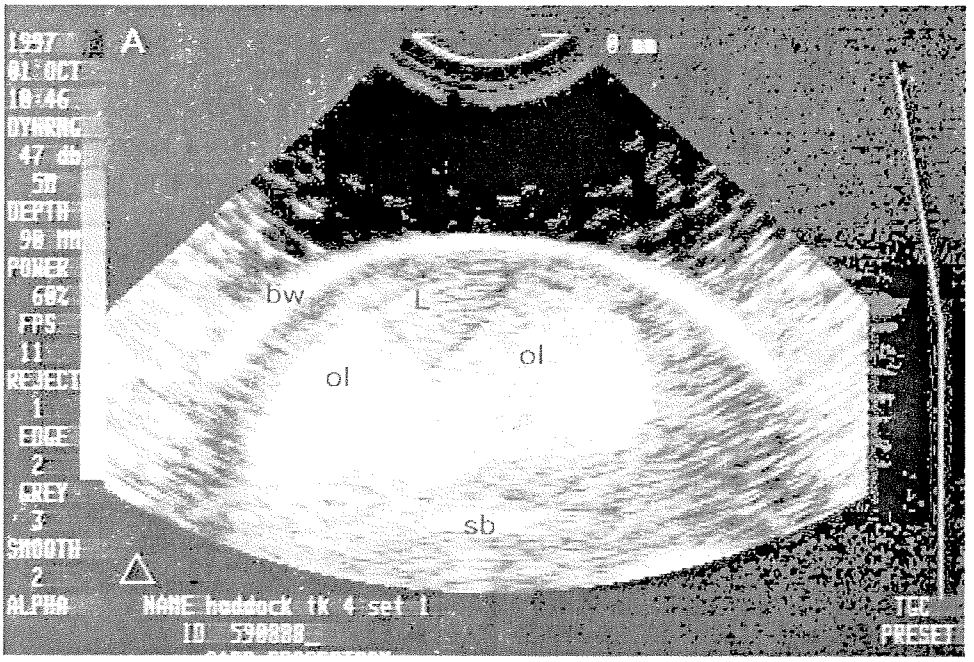

Fig. 2. Ultrasound image of female haddock (*Melanogramus aeglefinus*). ol, ovarian lamella; sb, swimbladder; bw, body wall.

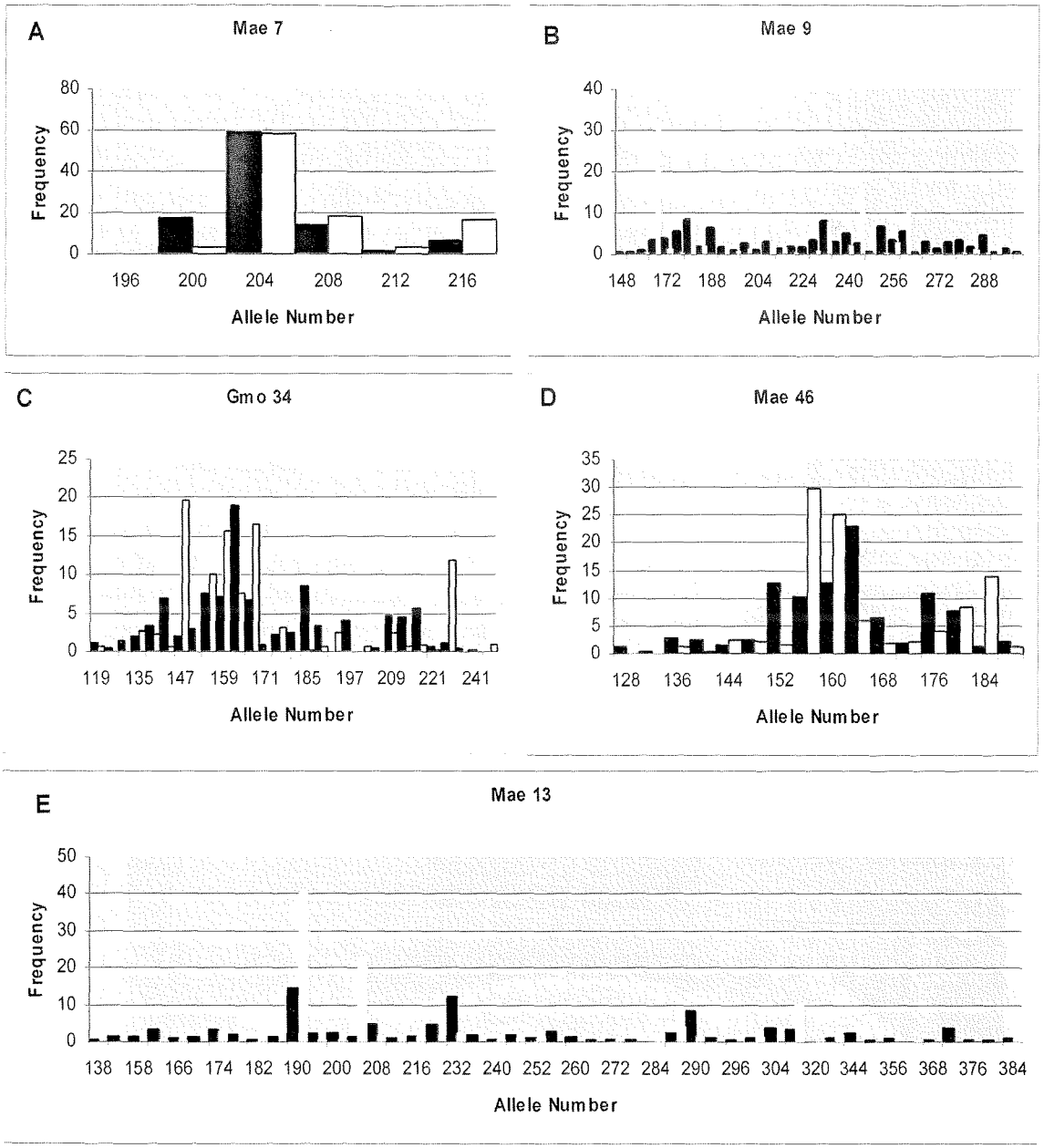

Fig. 3. Comparison of allele frequencies in each population (CZRI- white bars, SABS-black bars). A, Mae-7; B, Mae-9; C, Gmo34; D, Mae-46; E, Mae-13)