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REPORT OF THE WORKING GROUP ON GENETICS

Umeå, Sweden, 29-30 May 1985

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1. PARTICIPANTS

The ICES Working Group on Genetics met in Umeå, Sweden, 29-32 May 1985. The following members, appointed by the national delegates, were present:

M.L. Koljonen, Finland
L. Nyman, Sweden
G. Nævdal, Norway (Chairman)
R.L. Saunders, Canada

The following observers invited by the delegates from Sweden, were present:

L-O. Eriksson
T. Eriksson
J. Nilsson
M. Rasmusson
O. Ring
A. Weimarck

2. TERMS OF REFERENCE AND ITEMS FOR THE MEETING

At the 1984 Statutory Meeting it was decided (C.Res.1984/12:32).

The Working Group on Genetics (Chairman Dr. G. Nævdal) will meet in Älvkarleby, Sweden, from 4-6 June 1985 to:

- (i) continue work on a proposed international registry of cultured strains and stocks of finfish and shellfish to be published as an ICES Cooperative Research Report,
- (ii) prepare a report on the genetic bases of resistance to diseases, and the utilisation of such in breeding programs,

- (iii) update report on aquaculture genetics activities in different countries, on population genetics of resource species, and on basic genetics studies of relevance to aquaculture,
- (iv) further consider new approaches in aquaculture genetics.

Because the Symposium "Aquaculture in Sub-arctic Areas" was arranged in Umeå, Sweden, the days 4-7 June, the meeting dates of the Working Group were changed to 29-31 May and the meeting was moved to Umeå in order to simplify participation in both the Symposium and the meeting of the Working Group.

Based upon the terms of reference and a request from the Canadian member, the following Agenda was put up:

29 May: 1000 a.m. Welcome and introduction of participants.

Introductory remarks by chairman.

Short review of earlier reports and activities of the Working Group.

Information on other symposia and meetings of interest to the Working Group.

0130 p.m. Reports on activities in aquaculture genetics, population genetics and basic genetics in ICES member countries.

Effect of intensive salmonid culture on natural populations

30 May: 0900 a.m. Establishment of national and international strain registries.

0130 p.m. Genetic variation in resistance to diseases and utilization of such resistance in aquaculture. New approaches in aquaculture genetics. Strategy and further work of the Working Group. Recommendations.

31 May. Excursions to Norrby Laboratory (new) and a production hatchery (for stocking the Ume/river)

3. GENETICS RELATED TO MARICULTURE AND MANAGEMENT OF NATURAL RESOURCE POPULATIONS IN THE ICES MEMBER COUNTRIES.

As a first approximation the Working Group in 1981 compiled an account of the activities related to mariculture in the ICES member countries. These activities include quantitative genetics on aquaculture species applying techniques of selection and hybridization as well as more basic research and technique of potential importance to mariculture genetics. This account was updated in 1983.

At the meeting in Lowestoft in 1983 a first account concerning activities on genetics of national populations was compiled.

This year an updated account covering both aspects were prepared. The close relation between genetics of national populations and aquaculture genetics, especially concerning sea-ranching and enhancement program, made such combination necessary. Genetics on freshwater fish is included in so far as it provided models for marine species.

In the case of those countries represented in the Working Group, verbal or prepared reports were presented summarizing national activities in the field of aquatic genetics. For countries not represented the chairman asked the delegates (through the General Secretary of ICES) about such activities in their respective countries.

The activities reported are listed in Appendix I.

4. CONSIDERATIONS OF EFFECT OF INTENSIVE SALMONID CULTURE ON NATURAL POPULATIONS.

This item has been considered in more general terms at the two preceeding meetings of the Working Group during which actual and potential genetic influence of mariculture on natural populations was throughly discussed. Recommendations for use of non-reproducing strains or sterile animals when introducing populations into new areas and for careful evaluation of

ecological demands of such populations in order to avoid competition with endemic populations, have been put forward (see ICES/CM 1984, F:4). This year the following more specific question was raised by the Canadian member, Dr. R.L. Saunders:

"To what extent does introgression resulting from stock transplant for aquaculture reduce or affect fitness of natural populations? What would be a suitable experiment to measure such introgression and its effect?"

The immediate background for the questions is the suggestion of introducing Atlantic salmon eggs from Europe for cage culture purposes in Canada due to possible lack or inadequate numbers of eggs from natural or cultured salmon in the area.

The Working Group recognized the questions and pointed to some literature references which could throw light upon the problem, especially the proceedings from the Stock Concept International Symposium, Allistone, Ontario, 1980 (Can.J.Fish.Aqua.Sci. 38(12)). Examples of escape of cage reared salmon are numerous, (among others in Norway), but introgression of genes into endemic natural populations is rarely documented. Altuhkov (1981) mentions examples of decreased production of both the original natural population and introduced population when non-endemic populations are introduced. Evidently, the difficulty of measuring fitness is a major reason for the lack of data in the field, and this also limits the possibility of designing experiments to measure effects of introgression.

The problem has also been recognized by the "Working Group on Fish Gene Cooperation in the Nordic Countries" (Arbeidgruppe for genbanksamarbeid i Norden) under the Nordic Council. A research project has been outlined to study effects of escape of cultured fish on natural populations of Atlantic salmon, sea trout and arctic char. The outline of the plan was used as a working paper for the Working Group on Genetics. According to the plan, stock hybridization and gene introgression will be measured by biochemical gene markers, and, if possible, fitness will be measured as survival of progeny resulting from stock

crosses compared with "pure" stocks in successive generations. The experiments will be carried out in river systems where all ascending and descending fish can be controlled.

The Working Group considered the plans valuable and recommends that the studies be carried out as soon as possible. In the meantime, a conservative attitude to transfer of stocks, especially over long distances, is recommended to avoid potential reduced fitness and introgression of genes from such stocks into indigenous stocks. These conclusions are summarized in recommendations nos. 1 and 2.

5. NATIONAL AND INTERNATIONAL STRAIN REGISTRIES.

This topic was discussed at the Working Group meeting in 1984, and it was recommended that the ICES member countries should develop strain registries of fish and shellfish, and forward information on strains to the Working Group for inclusion in a proposed international strain registry. The Trout Strain Registry prepared by H. Kincaid and published by the National Fisheries Center-Leetown, W.V., U.S. Fish and Wildlife Service, was used as model. In this registry the following definition of "strain", also accepted by the Working Group, was used:

Strain - A population that exhibits reproducible physiological, morphological or cultural performance characteristics significantly different from other populations, or from other stocks derived from such populations and maintained thereafter as a pure breeding population. The following qualifications should be met for populations accepted as strains:

- (1) The population has been separated from its original source by at least 2 breeding generations.
- (2) The population differs in 1 or more performance trait or some other outstanding characteristic from its original source.

- (3) The population is sufficiently large to make a portion of it available on request, or at least some late-stage larvae from it.

At the 1985 meeting, responses with tentative national strain registries were received from Finland, Sweden and Norway in addition to the original Trout Strain Registry covering USA. The tentative responses from the Nordic countries are included as Appendix 2.

At the recent International Shellfish Conference, La Rochelle, France, it was recommended that a shellfish inventory be prepared under auspices of the Working Group on Technology, Growth, Employment, established at the '82 Versailles Conference (World Mariculture Society). It remains to be learned whether or not ICES and the Versailles Working Group can cooperate on a common inventory.

The definition of "strain" used by Kincaid (1982) and accepted by the Working Group on Genetics, evidently has caused some difficulties in establishment of national strain registries. The comprehensive and very useful list from Finland was characterized more as a broodstock registry than a strain registry. For such reasons the Working Group found that for national strains each country should use its own definition of "strain" in order to cover as many characteristics as possible and to give complete accounts of what really exists as culture strains in each country. For inclusion in an international registry, a common definition of "strain" should be used so that we can be more selective when this registry is established.

The purposes and intentions of an international strain registry were also considered briefly during the discussion. The benefit of national strains was clearly seen with respect to sensible planning of choice of "strains" for culture and conservation work within each country or region. Moreover, such strains should allow useful comparison of fitness and performance of a given species being reared in different countries or regions within a country. However, it was also

stressed that establishment of strain registries should not encourage increased transfer of species and strains between countries. Such transfer is, at present, viewed as a dangerous practice both for genetic and disease points of view. The Working Group regrets the rather low responses which have been received to date from the member countries.

The discussions are summarized by recommendation 3 and 4, which concerns the national and international registries respectively.

6. GENETIC BASES OF RESISTANCE TO DISEASES.

The Working Group discussed this item tentatively at the meeting in 1984. The discussion this year was based upon working papers by Arlene Longwell and John Bailey, as well as information given in H. Kincaid: Trout Strain Registry, and otherwise published information.

Diseases still represent main problems both in fish and shellfish culture. Fish breeders have been concerned mostly with non-genetic means of disease control, prevention and treatment, and at the moment reasonably good controls of disease problems are achieved in fish farming. Epidemic diseases of oysters, and absence of any practical treatment for these on large scale, make shellfish culturists more dependent in the foreseeable future on genetic resistance to disease than fish (and farm animal) breeders. On the other hand, resistant strains of fish would be very helpful in reducing the need to use large quantities of antibiotics and drugs for disease control.

Some of the best examples of genetic variation in resistance to fish disease are referred to by Kincaid (1980) and the working paper by Bailey. Both of these are mainly concerned with furunculosis; Kincaid mentions several resistant strains. An example of resistance to vibriosis was published by Gjedrem and Aulstad (1974) who found significant variation between natural stocks of salmon, but low heritability on a within stock basis.

Through natural selection, the oysters (Crassostrea virginica) of Prince Edward Island, Canada, seem to have developed resistance to the Malpeque disease. Oysters (same species) of the U.S. coastal mid-Atlantic appear to be developing genetic resistance to the MSX disease. Haskin and Ford (1979) reported on the development of resistance to Minchinia nilsoni (MSX) mortality in laboratory-reared oyster stock in Delaware Bay (copy of the 1979 paper provided Genetics Working Group with this report). Importantly, the oyster is such a prolific invertebrate that its response to natural selection for resistance to an epidemic disease is, potentially at least, more favourable than in less prolific species. Recovery should occur sooner than possible in a less fecund animal.

Although the examples referred to show some potential in utilization of genetic resistance, a number of important questions are still to be answered.

- a) An important question, with particular reference to fishes, is whether or not resistant individuals and strains are carriers of the disease agents. From experiences with strain resistance to furunculosis in brown trout, it was found in the USA that such strains were carriers of the disease and constituted a threat to non-resistant strains and populations coming in contact with the resistant individuals.
- b) Does resistance to one particular pathogen also confer resistance to other pathogens and/or other genetic types of the same pathogen? In other organisms, it has been found that the resistance can be non-specific and represent general antibody response mechanisms or it may represent variation in tissue- and cell wall permeability.
- c) Mode of inheritance. In cases where resistance is dependent on polygenic inheritance, several generations of selection will usually be necessary to develop a high degree of resistance. With selection for or against a single gene, progress should be comparatively rapid. In

crop and farm animals both kinds of genetic control are found. At the moment we know little about aquaculture species, although polygenic inheritance is indicated in the known examples.

- d) Exposure of experimental stocks to pathogens is difficult to standardize. In oysters, natural selection has been applied for breeding for resistance to diseases. In fish, natural infections have been used for counting family differences in mortality rate. This method, however, suffers from the drawback of introducing systematic environmental variation due to differences in time and rate of infections. Controlled exposure to pathogens may be applied, but this requires particular laboratories for such studies, because the pathogens are supposed to be kept at a minimum in hatcheries and at research stations.

Family selection will be a reasonable procedure for improving disease resistance in fish.

- e) In plants as well as in farm animals, hybridization and back-crossing are applied to incorporate favourable traits (i.e. disease resistance) in the genotype of valuable species.

Such techniques may well be of interest in aquatic species as well. More sophisticated methods from the field of genetic engineering will possibly become available in the future, as for instance in vitro hybridization techniques.

The discussion is summarized in recommendation no. 5.

7. NEW APPROACHES IN AQUACULTURE GENETICS.

An account on molecular cytogenetics in relation to genetic variation, stock identification and new methods of genetic selection was worked out by Arlene Longwell, USA. This account is included as Appendix 3. In Canada (see Appendix 1, Canadian

account, p. 22-23) research is being conducted on the possibility of improving freezing resistance of Atlantic salmon by introducing from winter flounder the gene or genes involved in production of polypeptide antifreeze compounds. The introduced flounder genes have been identified in the salmon. It was pointed out to me that we do not know it is in the genome until the animals have been bred, i.e., it may be in somatic cells or cytoplasm but not necessarily in the germ cells, but at the moment it is not known whether or not and under what condition the genes will be activated or expressed.

The Working Group found the possibilities and the obtained results in this field very interesting, and of potential use both in aquaculture and for stock identification. However, the practical applications have barely begun to be considered, and research in this field is still to be regarded as basic. The group considers aquaculture species as well suited as pilot species for such research because immediate practical application if the basic results seem promising (recommendation no.6).

8. STRATEGY AND FURTHER MEETINGS OF THE WORKING GROUP.

The Working Group on Genetics has now been in operation for five years, with meetings each year. Originally a biennial schedule of meetings was proposed. The participation at the meetings has been reasonably good although some appointed members have not had travelling funds to attend any of the meetings. At this year's meeting, only four appointed members were present. This may have been because an international symposium on genetics in aquaculture was to take place in California a short time after the Umeå meeting.

In 1986 ICES will cooperate with EIFAC in arranging a symposium in France on aquaculture genetics, and a minisymposium on fish genetics will be conducted during the Statutory Meeting. For these reasons the Working Group does not find it appropriate to meet in 1986, but rather work by correspondence for completing the national and international strain registries (Recommendation nr. 7). It is likely, however, that Working Group members

present at the Symposium will be able to meet at least briefly to attend to business of the Group.

9. REFERENCES.

Altuhkov, Yu.P. 1981. The stock concept from the viewpoint of population genetics. *Can.J.Fish.Aquat.Sci.* 38: 1523-1538.

Gjedrem, T. and Aulstad, D. 1974. Selection experiments with salmon. I. Differences in resistance to vibrio disease of salmon parr (Salmo salar). *Aquaculture* 3: 51-59.

Haskin, H.H. and Ford, S.E. 1979. Development of resistance to Minchinia relsoni (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Mar. Fish. Review*, Jan.-Feb. 1979: 54-63.

Kincaid, H. 1981. Trout Strain Registry. U.S. Department of the Interior. FWS/NFC-L/81-1. 118 pp.

10. RECOMMENDATIONS.

1. Transfer of non-indigenous species or strains should for the present be done only under carefully controlled experiments until more is known about possible genetic risks to indigenous strains. Such experiments should be conducted under strict quarantine because of the attendant dangers of introducing diseases and considering different susceptibilities to disease among diverse strains and mutant disease strains.
2. The Working Group on Genetics encourages the conduct of experiments to study the consequences of introducing foreign strains where they could hybridize with indigenous stocks or strains to determine whether or not the groups interbreed and if introgression takes place. Such studies should include measure of fitness of the resulting popula-

tions after genetic mixing. The proposed study by the Gene Bank group under Nordic Council to answer this and other questions is endorsed by the Working Group.

3. ICES member countries should be encouraged to continue to work on national strain registries, which should cover as many characteristics of the strains as possible. In view of the definitions of strains which differ among countries and even among workers in a single country, each country should report what criteria it uses to define strains being used or developed within the country.
4. ICES member countries should be encouraged to compile lists of strains for inclusion in an International Strain Registry. For inclusion in the International Strain Registry the definition and criteria of a strain in Trout Strain Registry, by H. Kincaid, should be applied. Cooperation with other international groups should be considered.
5. Geneticists and pathologists should be encouraged to cooperate in studies of variation in resistance to diseases in fish and shellfish to extend understanding of such variation and to make possible application of this information in aquaculture.
6. Studies in the rapidly developing field of genetic engineering, i.e. molecular cytogenetics, should be encouraged with aquatic animals for potential application in stock identification and aquaculture.
7. The ICES Working Group on Genetics should work by correspondence in 1986 with the main aim of completing national and international strain registries.

APPENDIX 1

CURRENT AND PLANNED GENETIC STUDIES RELATED TO MARICULTURE AND NATURAL RESOURCE POPULATIONS IN THE ICES MEMBER COUNTRIES

This document incorporates material solicited from individuals or groups in ICES member countries conducting studies in genetics with reference to aquaculture or natural resource populations. It updates information solicited for the meetings of the Working Group on Genetics in 1981 and 1983. From countries not represented in the Working Group, information has been given by the national delegates.

1. BELGIUM

No information.

2. CANADA (report compiled by R.L.Saunders)

Abstract

This document incorporates material solicited from individuals or groups in Canada conducting studies in genetics with particular reference to aquaculture. It updates information solicited for the last such report to the ICES Working Group on Genetics in May 1983. It is planned that this report be updated occasionally, incorporating any appropriate activities not mentioned this year and new studies planned or started between such updates.

Résumé

Le document regroupe les renseignements demandés aux particuliers ou groupes canadiens menant des études sur la génétique dans le domaine de l'aquiculture. Le document met à jour les renseignements recueillis pour le rapport précédent au groupe de travail sur la génétique du CIEM préparé en mai 1983. On prévoit mettre à jour le rapport de temps à autre en faisant état de toute activité connexe non mentionnée cette année ainsi que de toute nouvelle étude prévue ou entreprise entre la publication des mises à jour.

Aquaculture Genetics Program - Dalhousie University, Halifax, Nova Scotia -
G. F. Newkirk

Response to selection in an inbred stock of European oysters

In 1980 lines of the European oyster, Ostrea edulis, selected for live weight were produced including high and low first generation selected lines and high selected second generation lines. Unselected control lines were produced from both the stock from which the selected lines were derived and from a recently imported stock. The lines were replicated in two sets. The means of the lines after three growing seasons showed a response to selection over the two generations in spite of the inbreeding which had

previously been demonstrated in the stock. The unselected lines of the recently imported stock were about 25% heavier than the second generation selected lines. These results underscore the importance of evaluating and utilizing stock differences in aquaculture breeding programs. The selection program for improved oysters for culture in Nova Scotia is continuing with this new stock. Crosses with the old selected stock have also been done to incorporate the selected genes into a synthetic stock.

Fisheries and Environmental Sciences Division, Biological Station,
St. Andrews, N.B. - D. E. Aiken and S. L. Waddy

Development of broodstock for lobster culture

Early efforts to develop a domesticated strain of lobsters that would be amenable to culture were hampered by limited knowledge of lobster reproduction and problems in broodstock management. Progeny could be routinely obtained from wild parental stock, but not from animals raised in a culture system. Most of these problems have been brought under control in recent years, and the technology is now available for obtaining progeny from lobsters that have been hatched, reared to maturity and mated under culture conditions.

Lobsters hatched and reared in a culture system are subjected to selection pressure, and those that survive and grow rapidly under crowded conditions at relatively high temperature are considered better adapted. In the Lobster Culture Facility at the Biological Station, St. Andrews, New Brunswick, more than 200 cultured broodstock were selected from the many thousands that grew through the system in the past 10 yr. Selected F2 descendents of these animals now number approximately 400, some of which are approaching maturity at 60 mm carapace length.

In addition, a parallel selection program is being conducted with unusual color strains. Emphasis is placed on the red and blue variants, although white and yellow have also been involved. The program currently contains maturing F2 RxN progeny, and ovigerous F1 animals from RxR and RxB crosses.

Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews,
N.B. - J. K. Bailey, J. M. Anderson and C. B. Schom

The Salmon Genetics Research Program has been ongoing since 1974 with breeding studies initially using several wild stocks of Atlantic salmon. The long-term objectives of the program are to develop technology for the production and evaluation of strains of S. salar which are specifically adapted for sea ranching and cage rearing. Present breeding programs are using sea-ranched returns, cage-reared adults and wild Saint John River stock spawners to establish four select lines and accompanying control lines.

Sea ranching

During 1984, further progress was made towards meeting the goals of the SGRP. The 1984 year-class establishes select Line D, the last of four Atlantic salmon lines being developed with traits appropriate for aquaculture. The broodstock for this line were obtained from the Saint John River.

The 1984 smolt release program was profoundly affected by a furunculosis epidemic which began in May. The 1985 releases will also be affected. Both the 2+ smolts of Line A and the 1+ parr which would have produced the 2+ smolts of Line B were lost to the disease. These fish were destined to produce the grilse broodstock required to advance both lines. Fortunately, samples from both lines had been transferred to sea cages before the epidemic and the genetic information was not completely lost. Broodstock for these lines will be selected from the caged fish in 1985 and 1986.

The number of returning salmon was very low in 1984. A total of only 64 salmon, including 53 grilse and 11 2-sea-winter salmon were recovered from smolt releases made in 1983 and 1982; respectively. The reasons for this return rate of much less than one percent are unknown.

Genetic analysis of furunculosis resistance

A furunculosis epidemic during May, 1984, made it possible to estimate the genetic component of furunculosis resistance. Among 42 full sib families in the same tank, mean survival ranged between 18% and 98%. This yielded a heritability estimate of 0.32 ± 0.06 . The genetic component is significantly greater than zero and indicates that selection for this trait is feasible.

Genetic analysis of freshwater growth

In 1978 and 1979, full and half sib families were produced by hierarchical matings within two and three stocks of Salmo salar, respectively. Under standardized hatchery conditions there were no significant differences in length or weight among the stocks examined at 3, 6 or 15 mo post hatch. Half sib heritability estimates for length and weight were low to intermediate (0.1 to 0.4) in 1978 and intermediate to high (0.4 to 0.9) in 1979. Differences among stocks and levels of domestication between years are suggested as possible explanations for such variation. Genetic, environmental and phenotypic correlations between length and weight at the three sampling times were positive and high (>0.7). The results suggest that relatively rapid genetic gain for these traits is possible if selection intensities are high.

Comparison of sea ranching and cage rearing

Full sib families of Salmo salar were divided into two groups. One portion was sea ranched and the other was placed in floating net pens. The ratio of grilse to larger salmon was significantly different between the cage and sea ranch treatments (0.5 and 8.3, respectively). This difference was attributed to environmental factors. Percent survival among those families which returned a minimum of two individuals in both environments showed a moderate, positive correlation ($r = 0.45$, $p < 0.1$) between treatments. Selection for increased survival in the cage environment would be expected to produce a correlated response among released salmon.

Acid rain

The study on the genetic component of low pH resistance in Atlantic salmon was concluded in 1984. Twelve hundred 0+ parr from six families were challenged. As in previous years, there were family-specific differences in survival time. In addition, a differential response was observed between mature and non-mature parr. The smaller mature parr tend to survive longer.

Stock Assessment and Genetics Unit, Research Section, Fisheries Branch,
Ontario Ministry of Natural Resources, P.O. Box 50, Maple, Ontario -
P. E. Ihssen

Genetics of growth in rainbow trout

This project has been conducted in cooperation with G.W. Friars and Laura R. McKay of the University of Guelph. Diallel crosses were used to estimate genetic components of variation for growth rate of fry, fingerlings, and yearlings. Genetic correlation between growth rate at different ages and at different temperatures was determined. Growth rates of different genetic stocks were compared. The laboratory work for this project was completed in 1984/85.

Hybrids, gynogenetic triploids of brown, brook and lake trout

Survival and growth of hybrids and gynogenetic triploids of these species is investigated. Initially, the emphasis is on studying the early survival (eggs, fry) and cytogenetic make-up of such hybrids and triploids. Methods are tested for genetically improving the viability of the brook x brown trout hybrid. The long-term objective is to assess such fish for use in aquaculture and put-and-take fisheries.

Selective breeding of rainbow trout

Rainbow trout have been bred by disruptive selection for high and low temperature tolerance. Tests are being conducted to determine whether the high and low tolerant strains are also genetically different for other physiological or biochemical characters. Growth rate experiments will be conducted in 1985/86 to test the hypothesis that the two strains for which the temperature tolerance has been shifted up or down by selection have a corresponding shift in their optimum temperature for growth.

Genetic impact of fish culture, domestication

The genetic variability of wild and semi-wild stocks of game fish that are exploited for various fisheries programs is assayed. Electrophoretic, morphological, cytogenetic and ecological methods are employed. Brood stocks and production lots derived from these stocks are monitored for possible adverse genetic effects associated with such programs. Loss of heterozygosity and changes in electrophoretic allelic frequencies are estimated.

Freshwater Institute, Dept. of Fisheries and Oceans, Winnipeg, Manitoba -
G. B. Ayles, M. H. Papst and M. Giles

Growth of Arctic charr

Genetic studies relevant to aquaculture are conducted by the Fish Production Systems Research Project; studies are primarily conducted at the Rockwood Experimental Fish Hatchery. Since 1981 an emphasis has been placed on studies related to the culture of Arctic charr. The growth performance of various strains of Arctic charr is being tested under different rearing conditions. Six strains of Arctic charr are maintained at the Rockwood facility. With the development of hatchery reared broodstock, an Arctic charr selection program has been initiated. Studies to determine the genetic basis of early male sexual maturation, dwarf individuals and cold water fast growing individuals, have begun. Estimates of genotype - environment interactions have been made for rainbow trout and are being made for Arctic charr.

Hemoglobin development in Arctic charr

The ontogenetic development of hemoglobin in Arctic charr (Nauyuk Lake, anadromous strain) has been established in embryos, 1 and 2 year fry and adults. Preliminary analysis of hemoglobins from other strains of Arctic charr at the Rookwood Hatchery has been initiated. Three phenotypic variants, incorporating 10-12 hemoglobins have been identified in Nauyuk Lake charr and an analysis of changes in their frequency in the different age classes initiated. Studies to assess possible differences in the physiological capabilities of the different phenotypic variants will begin in 1986/87. Results of this work will be used in the development of Arctic charr brood stock programs.

Fisheries Research Branch (Pacific Region) Fish Culture Research Section,
West Vancouver Laboratory, Dept. of Fisheries and Oceans,
W. Vancouver, B.C. - E. M. Donaldson, G. A. Hunter, T. J. Benfey,
I. I. Solar and I. Baker

Production of all-female stocks of Pacific salmon

The studies on the production of all-female stocks of Pacific salmon, mainly concerned with the enhancement of depressed chinook populations, have continued. Approximately 420 thousand all-female chinook for release have been produced at the Capilano Hatchery in 1983 and 1984. One hundred thousand of these fish were re-masculinized by hormonal treatment and will produce "female milt" at their return to the hatchery. The monosex stocks were originally produced using "female milt" from genetic females masculinized with androgens. Monitoring of the tagged released chinook and evaluation of their impact in the fishery by future returns and increased egg-take will follow. Additionally, "female milt" has been used to assist fish farmers in the production of 750 thousand all-female chinook for netpen aquaculture in B.C.

Experiments on the use of different steroids for the masculinization of female chinook including naturally occurring androgens have also been carried out.

Production of sterile Pacific salmon

Sterilization treatments have been conducted with coho and chum salmon at the production level. Approximately 400,000 sterile coho have been released from Capilano and Big Qualicum Hatcheries, and 150,000 sterile chum were produced at Thornton Creek Hatchery. Data obtained from these and previous releases are expected to reinforce existing indications of a positive impact of sterile salmon to the fishery.

The technique is also being applied at private fish farms to sterilize approximately 550,000 coho and chinook salmon for netpen rearing. In collaboration with the province of B.C., studies are also being conducted on the production of sterile land-locked sockeye and rainbow trout for release into inland waters.

Several benefits are expected to be realized from the releases of sterile hatchery stocks, among them: eliminate or delay sexual maturation and return migration of a fraction of abundant hatchery stocks. This will reduce production of "jacks" and could mean a substantial contribution to the commercial and sport fishery. Additionally, the sterile hatchery fish could contribute to alleviate fishing pressure on wild stocks and reduce the genetic impact of interbreeding between hatchery and wild stocks.

Chromosome set manipulation

The use of chromosome set manipulation to control reproduction in Pacific salmon is currently under study. The induction of polyploids (triploid) and cloning by gynogenesis are considered promising techniques to produce sterile fish and for maintaining superior genotypes, respectively. Heat shock, and hydrostatic pressure shock have been used to induce triploidy in pink, coho and chinook salmon. Further to improving the technique to enhance treatment effectiveness and triploid survival, studies will be conducted to monitor gonadal development and radioimmunoassay will be used to develop profiles of plasma steroid hormones in triploid salmonids.

Production and performance of Pacific salmon hybrids

Satisfactory fertilization rates, survival and growth have been obtained in female coho x male chinook hybrids. Preliminary results suggest that the above mentioned hybrids may have better grow-out performance than their reciprocal hybrids and the intraspecies control crosses. Further assessment of the hybrids' performance could determine their merits for aquaculture.

Sex control in rainbow trout

Success has been achieved in the production of functional phenotypic males (genotypic females) in rainbow trout. All female offspring have been produced. The technique will be available to enhance stocks of wild rainbow trout in British Columbia inland waters and, using domesticated stocks, to produce all-female rainbow trout for freshwater and sea culture. Additionally, a higher percentage of sterility than any previously reported has been obtained with hormonal treatment. All triploid (100%) rainbow trout have also been produced with both heat and hydrostatic pressure shocks. Survival and growth of triploid domestic rainbow trout reared for two years has been found to be comparable with diploid controls.

Fisheries Research Branch (Pacific Region), Pacific Biological Station,
Dept. of fisheries and Oceans, Nanaimo, B.C. - B. E. Ridell and
R. Withler

Population genetics

In light of the lack of information on genetic variation between salmon populations in British Columbia when the genetics program was initially formed (May 1981), a substantial amount of effort has been expended on electrophoretic surveys of all five Pacific salmon species. The University of British Columbia has investigated coho salmon (contact Conrad Wehrhahn) and the other four species have been studied by the Fisheries Research Branch. More detailed studies on changes in gene frequencies in hatchery and proximal, natural populations (referred to in C.M.1983/F:18) are continuing, as are the studies on heritabilities of life-history traits. Changes in gene frequencies resulting from domestication are now being studied in chum and chinook salmon, and we expect to continue this monitoring for several more years. Studies up-to-now have only provided baseline data on natural populations for comparison with hatchery produced fish.

New programs include: an evaluation of using restriction enzymes on mitochondrial DNA to measure genetic variation between salmon populations and possibly use any differences for stock identification (principle investigator - Ruth Withler, in collaboration with Dr. A. Beckenbach, Simon Fraser University); and behavioral genetic studies of sib-recognition in coho salmon and mate selectivity by sockeye salmon (conducted by P.D.F's and a Ph.D. student).

Quantitative genetics

- i) Strain performance comparisons: To assess the value of screening numerous populations of coho and chinook salmon for their suitability to mariculture, pilot studies involving several populations of each species are on-going. Both species will be transferred to sea water in June 1985. Our only comparison to date is for freshwater growth of coho. All five populations of coho show very similar freshwater growth performance. Expected completion date for coho is Oct. 1986 and for chinook is Oct. 1987.

- (ii) Early maturity in male coho salmon: A genotype-environment interaction study has been designed to evaluate the determinants of early male maturity in coho salmon. This experiment will be completed in Nov. 1985 and will evaluate female and male parent effects as well as the effects of enhanced and restricted environments during freshwater rearing (enhanced environments will result in smolts being almost double the size of their sibs in restricted environments).

- (iii) Selection for increased body size at maturity in coho salmon:

To estimate the realized heritability of body size at maturity in coho salmon and to test the feasibility of conducting genetic selection in a production hatchery, we initiated a selection program in Nov. 1983. This program involves two generations (through Nov. 1989) of positive selection plus a random-mating control line in the hatchery, and a two-way selection experiment plus a control line are simultaneously in net-pens. Hatchery-reared fish will be released as smolts but the latter experiment will provide more control over the marine life phase by maintaining the lines in net-pens.

- (iv) Genetic basis for red versus white flesh in chinook salmon

Following two years of preliminary studies which indicate that there is a genetic basis to flesh colour in chinook salmon, Ruth Withler has produced an 8 x 8 diallel cross to study the genetic parameters involved in regulating the trait's expression. Completion date of this study is expected to be Oct. 1986.

- (v) Broodstock development

During the development of broodstock for mariculture, we will be testing the various chinook strains in (i) above in three additional fish farm sites to investigate strain x farm interactions. Further, in order to evaluate the merits of allowing Atlantic salmon on the Pacific coast of Canada, we are in collaboration with C. Clarke, importing Atlantics from Scotland. The performance of Atlantic salmon will be compared with coho and chinook salmon reared in the same environment.

Marine Science Research Laboratory, Memorial Univ. of Newfoundland,
St. John's, Newfoundland - M. A. Shears, G. L. Fletcher (Memorial Univ.), C. L. Hew (Dept. Biochemistry, Hospital for Sick children, Toronto, Ontario) and P. H. Davies (Dept. of Biochemistry, Queen's Univ., Kingston, Ontario).

Genetic manipulation to produce freeze resistance in Atlantic salmon

Atlantic salmon and other salmonids freeze to death when the water temperature declines below -0.7°C . Since seawater temperatures below this value are the norm in Atlantic Canada, the culture of salmon in marine cages is restricted to a very few areas where the water temperatures do not normally fall below 0°C . However, if a breed of salmon capable of surviving the relatively brief period of sub-zero winter water temperatures could be developed, vast areas of protected coastal waters would be ideal for their commercial culture.

Many other fishes such as the winter flounder thrive in this freezing environment by producing antifreeze proteins/polypeptides in their serum. We have already isolated and characterized both the antifreeze polypeptides and their genes. It is proposed to improve the freezing tolerance of Atlantic salmon and other salmonids. The antifreeze protein gene(s) isolated from the winter flounder are being introduced into the genome of the recipient salmonids by gene transfer using a micro-injection procedure. The presence and expression of the injected gene(s) is being examined by various biochemical and molecular biological techniques. The fish containing the transplanted antifreeze gene(s) will be cross bred to determine its heritability.

Huntsman Marine Laboratory, St. Andrews, N.B. - B. D. Glebe and E. Verspoor

Atlantic char - Atlantic salmon hybridization and chromosome engineering study

Three char stocks (two anadromous and one landlocked) are being compared for their adaptability to hatchery and sea-cage rearing in New Brunswick. To date, fry from all stocks have initiated feeding at temperatures as low as 4°C and survival at temperatures between $4-12^{\circ}\text{C}$ during the swim-up and first feeding phase has been excellent.

Heat shock induced triploidy and hybridization (char-salmon) are being investigated as mechanisms for combining the cold water adaptation of char with the marine growth and seawater survival of Atlantic salmon when reared in sea cages. Hybrid growth was superior to that of pure salmon. However, a vertebral abnormality in the hybrid was apparent. The influence on smoltification and sea-water adaptability of the extra maternal chromosome set in triploid hybrids will be evaluated this spring.

Investigations into the biochemical genetics of the Atlantic salmon

In 1984, studies were carried out into genetic variation of blood and tissue proteins in Atlantic salmon populations in eastern Canada. Three aspects of the work are of interest to aquaculture: survey of populations in rivers to catalogue existing protein variation for potential use as genetic stock markers; the identification of a sex-linked serum protein which can be used to non-destructively identify the sex of immature and mature salmon; and the assessment of the developmental effects of genetic variation at a gene locus controlling the expression of the enzyme phosphoglucomutase in the liver. Genotypes at this locus in the rainbow trout show significant differences in the growth and development time of embryos. This work is continuing in 1985 at the Department of Fisheries and Oceans, St. John's, Newfoundland.

Fish Breeding Research, Dept. of Animal and Poultry Science, Univ. of Guelph, Guelph, Ontario - G. W. Friars, L. McKay and J. K. Bailey

Genetic analysis of growth and carcass composition in rainbow trout

Maternal effects in rainbow trout dissipate with age but interfere with selection for market weight before 1 yr of age.

Determination of optimum selection criteria in sea-ranched and cage-reared Atlantic salmon

The objective of this study is to demonstrate the relative efficacies of selection index and independent culling for breeding programs involving domestic Atlantic salmon in eastern Canada. The breeding objectives are restricted to the improvement of three traits which are important in both sea ranching and cage culture operations. The three traits are 6-mo parr length, percent 1-yr smolts and length after 15 mo at sea. Both individual and within family systems of selection are examined for each type of operation. Computer simulation techniques are used to generate experimental, multivariate normal distributions of the three traits under selection.

3. DENMARK

No information.

4. FINLAND (report compiled by Marja-Liisa Koljonen)

Finnish Game and Fisheries Research Institute, Fisheries Division,
P.O. Box 193, SF-00131 Helsinki 13, Finland

- 1) The enzyme gene variation of salmon stocks in Finland,
M-L. Koljonen

The enzyme gene variation in the Finnish salmon stocks has just been completed. The variation was studied in 8 enzymes and 25 enzyme loci. The material consisted of 20 samples taken from different salmon populations during 1981-83, altogether 1314 specimens. Statistically very significant differences were found between all salmon stocks of different origin. On average, the hatchery stocks showed less genetic variation than the natural stocks.

- 2) The enzyme gene variation of rainbow trout stocks in Finland,
M-L. Koljonen

While a nation-wide fish-breeding program was worked out for rainbow trout, a study was made of the enzyme gene characteristics of the cultured rainbow trout stocks. The amount of genetic variation and differentiation of the stocks were determined by studying nine enzymes and 20 gene loci.

The autumn spawning rainbow trout was genetically most different. The genetic variation was greater in commercial stocks than in the stocks from the state fish culture station, which in their turn tended to differ more from each other than did the commercial stocks.

3) The enzyme gene variation of brown trout stocks in River Tornionjoki, M-L. Koljonen

Since the status of the sea trout in The River Tornionjoki is now threatened and it is planned to extend its farming, the enzyme gene characteristics were studied in fingerlings caught by electric fishing in different tributary rivers. The samples were taken in 1983 and 1984. The analysis of the material will be finished in 1985.

4) The enzyme gene variation of whitefish stocks in The Saimaa lake system, M. Heinonen and M-L. Koljonen

An electrophoretic study on the whitefish stocks in the Saimaa lake system was started in 1984.

5) Brood stock register, I. Kallio

A register has been created of the brood stocks at the State fish culture stations of the Finnish Game and Fisheries Research Institute. The register contains the following information from 10 fish culture stations: species, stock, establishment year, number of individuals, origin, use, culture record and number of hatchery generations. The register covers 15 species. The following cultured stocks have been registered: 3 salmon stocks, one land locked salmon stock, 5 sea trout stocks, 9 brown trout stocks, 3 non-migratory brown trout stocks, 5 rainbow trout stocks, over ten whitefish stocks, 4 char stocks and 2 grayling stocks.

6) State of stocks of Finnish migratory fishes, I. Kallio

A study was made of the Finnish migratory fish species for which information is available about natural stocks and the State brood stocks. The study included salmon, land locked salmon, sea trout, brown trout, non-migratory brown trout and grayling. The state of the natural stocks were evaluated from the fry production in the spawning grounds and from the data on the catches. The state of the brood stocks were evaluated from the amount of brood fish and number of station generations. The study showed that most brood stocks had been built up from rather few individuals.

7) Plan for a fish breeding station, O. Sumari, L. Siitonen and D. Linder

The decision has been made to build a fish-breeding station in Finland. The station will be administered by The Finnish Game and Fisheries Research

Institute. The general plan of the station was completed in 1984 and the building plan in the beginning of 1985. The fish-breeding station will first concentrate on breeding rainbow trout for food fish production. Other fish species will also be bred for food fish culture and for stocking. The year 1984 saw the completion of 3-year study on the variation within and between rainbow trout stocks concerning growth. This investigation, financed by the Academy of Finland, was undertaken with a view to planning and setting up a breeding program for rainbow trout.

8) Milt deep-freezing program, K.Nyholm and P.Eskelinen

Work on the fish gene bank, started in 1982, has continued at the Laukaa Fish Culture Research Station. Milt of brown trout from Central Finland and of Neva salmon has been stored at the station by deep-freezing.

9) Strain registers for brood stocks and natural stocks, I. Kallio and K. Ruohonen

A questionnaire and a method of registering were worked out in 1984 for a nation-wide inventory of the natural stocks. The inventory will be carried out in 1985. The planning of an EDP program for a register of cultured and natural fish stocks has been started at the Laukaa Fish Culture Research Station.

University of Joensuu

P.O. Box 111, SF-80101 Joensuu 10, Finland

Population genetic studies on fishes, J.Vuorinen and J. Piironen

The genetic study on salmonide populations has been continued at University of Joensuu. Six reports were published during 1984 and most of them dealt with the electrophoretic variation in the family Coregonus. About 3,000 fish from 40 populations were studied. Genetic changes during culture were investigated in one brown trout stock. As in some other studies, heterozygosity had clearly declined and some alleles had disappeared altogether during culture. In addition to the development of electrophoretic and sampling methods a diagnostic method has developed for living salmon and trout and their hybrids.

Milt deep-freezing studies, J. Piironen

Milt has been deep-frozen from land locked salmon, some trout stocks, rainbow trout, Inari char and whitefish from The Rivers Koitajoki and Pielisjoki. Besides being used in sampling and research, deep-frozen

milt has been used for 2-3 spawning periods to promote fertilization of the natural stock of land locked salmon. For some years already, milt of land locked salmon has been gathered from natural stocks for storage in a gene bank. Other methods are also being developed. Deep-frozen milt from whitefish has already been stored for over two years, mainly in order to experiment with genetic crossing. Milt from other species has been deep-frozen, mainly for the development of new methods, e.g. in order to shart the dependence between the consistency of the milt and its suitability for deep-freezing.

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5. FRANCE

An account was given in 1984 and enclosed as APPENDIX 6 of the report for that year.

6. GERMANY, Democratic Republic of ...

No information.

7. GERMANY, Federal Republic of ...

No information this year. Some information is included in the earlier reports.

8. ICELAND

No information this year. An account was given in 1983 and included in the report for that year.

9. IRELAND

No information this year. Comprehensive reports have been given at the Working Group meeting in 1981 and 1983.

10. NETHERLANDS

No information.

11. NORWAY

a) Aquaculture genetics

Experiments or quantitative genetics and selective breeding of salmonids are carried out at two institutions in Norway; The Department of Animal Genetics and Breeding, Agricultural University of Norway, Åa, and Institute of Marine Research, Directorate of Fisheries, Bergen. The practical experiments are carried out at research stations and at commercial fish farms. Mainly Atlantic salmon and rainbow trout are concerned.

At both institutions breeding experiments were started about 1970 to evaluate the potential for selective breeding in salmonids. First the genetic and phenotypic parameters for the traits of greatest economic importance were studied. The results have demonstrated that there exist a large genetic variation in growth rate and age at sexual maturity; a moderate amount of genetic variation in survival, resistance against diseases, meat quality characters and digestibility of food; and possibly a low genetic variation in condition factor. Genetic variation in flesh pigmentation is indicated both in rainbow trout and salmon.

Inbreeding and crossbreeding experiments have shown that some non-additive genetic variation seems to exist. However, it is still not clear what emphasize should be put on crossbreeding in a future selection programme.

An extensive selection programme is carried out on Atlantic salmon and rainbow trout by the Agricultural University at the research stations at Sunndalsøra and Averøya and in cooperation with several private fish farms. The base populations of Atlantic salmon was sampled from 40 different Norwegian strains. Each year about 200 full-sib families are tested from hatching

to maturation. Selection is based on individual performance and records from full- and half-sib families. The following characters are taken into consideration; growth rate prior to maturation, survival, meat quality and age at maturation. For rainbow trout, the selection programme is similar, and about 150 families are tested each year. The progress made during the first years of selection is very promising.

Experiments on induced polyploidy to obtain sterile salmon and rainbow trout are carried out at Sunndalsøra.

At the Institute of Marine Research, Bergen, similar experiments have been carried out since 1971, although in a smaller scale.

Due to IPN-virus in the material, the experiments had to be discontinued and started again with new material in 1978. A practical program for selective breeding of Atlantic salmon and rainbow trout are developed by the Norwegian Farms Association (Norske fiskeoppdretters forening) in cooperation with the research institutions. This work will be started in the fall of 1985 at a new station now built at Kyrksæterøra south of Trondheim.

New experiments on measuring genetic variation in food utilization (compared to food consumption) and variation in age at maturation caused by non-linear regression between genetical and environmental factors, are under way. Experiments with utilization of sex reversal of salmon in commercial scale have been carried out by a private firm (A/S Mowi).

Tests of aquaculture performance for strains of arctic char are carried out at the University of Tromsø.

b) Genetics of natural resource populations

At Institute of Marine Research, Bergen, population genetics studies on fish were started in the early sixties. Blood groups in cod, Gadus morhu, and blood protein polymorphism in cod, herring, Clupea harengus, and sprat, Sprattus sprattus, were identified and utilized for studies on population structure of these species. Enzyme polymorphism was used in a few cases. This work terminated in 1971 for capacity reasons.

The work takes place at the Norrby laboratory, in Kålarne Fisheries Research Station (in cooperation with the Freshwater laboratory) and at local south and North Swedish hatcheries and rearing units.

Appendix

1) The population genetics of Arctic char in Newfoundland and Labrador, Canada-----

In a joint venture between the Fisheries Research Branch of the Department of Fisheries and Oceans in St. John's, Newfoundland, and the Institute of Freshwater Research, Drottningholm, Sweden, Johan Hammar of the latter institute spent 10 months (June 1984 - April 1985) studying the species interactions, ecology and population genetics of landlocked, resident and anadromous populations of Arctic char in Labrador and insular Newfoundland.

Two landlocked, one resident and five anadromous populations were studied in Labrador, eleven landlocked and two anadromous populations were studied in insular Newfoundland. The electrophoretic analyses comprised an estimated 33 loci. In addition to this program Est - 2 frequencies were routinely screened in numerous populations from Newfoundland, Labrador and the Northwest Territories.

2) Population characteristics of anadromous Arctic char in northern Quebec, Canada-----

In a joint venture between the Makivik Corporation, Quebec, Canada, and the Institute of Freshwater Research, Drottningholm, Sweden, Rolf Gydemo of the latter institute performed blood genetic analyses on some 500 char caught in the Kovik, Payne and George rivers of northern Quebec. Preliminary results indicate that there may be two distinct sub-populations within the samples collected from Kovik River and George River. Only one population was indicated in the Payne River sample. Differential growth rates were observed for the two sub-populations in each case, however the relationship between the gene frequency mode and growth rates was inverted between the two river systems (data published in 1982).

3) The population genetics of landlocked and anadromous Arctic char in Greenland-----

In a joint venture between the Greenland Fisheries and Environmental Research Institute, the University of Copenhagen, and the Institute of Freshwater Research, Drottningholm, Sweden, Johan Hammar of the latter institute has performed electrophoretic analyses on 18 populations of char since 1981. Samples are taken from populations on the west, south and east coasts of the island.

4) Pattern and distribution of the Arctic char species complex in Iceland-----

In a joint Danish-Icelandic-Swedish research project on the biology of Lake Tingvallavatn the population genetics of the char stocks in the lake was evaluated by Rolf Gydemo of the Institute of Freshwater Research, Drottningholm, Sweden. In addition 44 more localities (rivers and lakes) were sampled and screened electrophoretically. Cooperation started in 1978 and continues. The data was published in 1983.

- 5) Production of landlocked Lake Vänern salmon for consumption.
Dr. Bengt Larsson, Department of Animal Breeding and Genetics,
Swedish University of Agricultural Sciences, Uppsala.
SJFR (research council).
- 6) The Lake Vänern Project - population genetic analyses of salmon
and trout stocks from the Lake Vänern drainage area: A model for
production and conservation. (Provisional title.)
Dr. Gunnar Ståhl, Institute of Genetics, Division of Population
Genetics, University of Stockholm.
FS (research council) and County Administration.
- 7) Effects of rearing on fish from a genetic and ecological per-
spective (salmon, trout and char).
Dr. Lennart Nyman, Institute of Freshwater Research and Experimental
Station, Kälmarne.
SJFR, FS (research councils).
- 8) Genetic and ecological classification of sibling species of
Arctic char.
Dr. Johan Hammar, Institute of Freshwater Research, Drottningholm
and Experimental Station, Kälmarne
"Internal" financing and SJFR (research council).
- 9) Strain registries of salmonid fish.
Dr. Lennart Nyman, Institute of Freshwater Research and Experi-
mental Station, Kälmarne.
SJFR and FS (research councils).

Projects related to fish genetics at the University of Umeå, Sweden

1. Genetic parameters and variation in salmon and Arctic char.
Prof. Marianne Rasmusson and Jan Nilsson, Institute of Genetics,
University of Umeå
Support: SJFR (research council)
2. Basic studies of Arctic char (Salvelinus alpinus L.) for
aquaculture purposes. Growth, life history and behaviour,
and rearing techniques are studied in four different popula-
tions of Arctic charr.
3. Basic studies of Baltic salmon (Salmo salar L.) for aquaculture
purposes. Growth, life history, behaviour, and smolting patterns
are studied in six different populations of Baltic salmon are
studied.
Doc. Lars-Ove Eriksson, FD Hans Lundqvist, Torleif Eriksson
Eva Brännäs and Bo-Sören Wiklund
Support: SJFR (research council)
4. Evaluation of risks to natural salmon populations of escapements
from sea cages and non-river based ranching.
Doc. Lars-Ove Eriksson
Prof. Marianne Rasmusson
Support: Fisheries Board of Sweden (Salmon research institute)

In 1978 similar work was started again, this time with the main emphasis on starch gel electrophoresis of tissue enzymes. Mainly herring and cod samples have been analysed with the aim of closer investigation of the population structure of these important commercial species in middle and northern Norway. Part of the analyses are based on fertilized eggs, newly hatched larvae and postlarvae. This work probably will continue, although, the financial support is somewhat uncertain. Studies on polymorphic systems of artificially reared cod larvae, cod in intensive aquaculture and cod used for stocking purposes are undertaken to study the stability of gene frequencies, correlation between gene of type and productive traits and relation between natural and stocked cod in certain areas.

At the University of Trondheim electrophoretic studies on tissue enzymes and hemoglobins have been carried out for several years, with the aim of studying the population structure of cod in a single fjord system, the mechanism of balancing the polymorphic systems and the influence of selective forces in such systems. A study is carried out on population structure and phylogenetic relationship between different species of gadoids and platessoides. Also a program on electrophoretic (electrophocusing) identification of fish eggs (mainly gadoids) are running.

At the University of Tromsø preliminary genetic analyses of capelin Mallotus villosus, and halibut, Hippoglossus hippoglossus, have been carried out.

At the University of Oslo population genetics of invertebrates, mainly krill, have been performed.

12. POLAND (report by Krzysztof Goryczko)

At Inland Fisheries Institute, Salmonid Fish Breeding Lab. experiments as follow have been started:

i. Sea trout - mating grilse and non grilse females and males (4 combinations). We are going to check the smoltification rate at 1+ and 2+ age, freshwater growth rate and the age of first maturity (in fresh water).

ii. Vistula river sea trout - start of selective breeding (for ranching purpose). Separate rearing offsprings of 30 selected (size as determinant) females. 20 of these females were tested for immunoglobuline and lysozyme level.

iii. In collaboration with prof. K. Bieniarz (Academy of Agriculture in Krakow) the sex manipulation tests with rainbow and brown trout, using estradiol and testosterone, were started.

13. PORTUGAL

No information.

14. SPAIN

No information.

15. SWEDEN (report compiled by Lennart Nyman)

The following list is very likely not complete, even though only half a dozen research institutes are involved in related activities within the country. Two known "cases" have not responded to the enquiry. To cut it short I have only included information on project titles and leaders, where the project is carried out and who finances it. As an appendix I have included an overview of research within the subject area performed by a Swedish national but in foreign countries. Preferably this list should be checked against the national lists of the ICES member countries mentioned.

1) The influence of temperature on sex differentiation in the European eel

Professor Karl Fredga, Institute of Genetics, University of Uppsala: cooperating institutes - Institute of Genetics at the University of Lund, Institut für Humangenetik und Antropologie der Universität Freiburg and the Institute of Freshwater Research, Drottningholm.
SJFR (research council).

2) Ecological and genetic studies on populations of Arctic char and grayling in environmentally affected waters.

Dr Hans Rytman, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala: cooperating institute - Institute of Genetics, University of Uppsala.
SNV (research council).

3) The population genetics of grayling.

Dr Håkan Jansson, Institute of Genetics, University of Uppsala,
FS (research council).

4) The growth pattern and its genetic variation in rainbow trout in varying production systems

Dr. Susanne Sylvén, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala.
SJFR (research council).

The four projects mentioned are part of a circumpolar study of Arctic char populations, actually providing material for a would-be international Arctic char registry by employing traits such as parasites, migration patterns, growth, spawning time, feeding habits, electrophoretic markers etc. The project is monitored within the ISACF Organization in cooperation with the Wild Salmonid Watch, and is presently encompassing data on stocks in Alaska, Canada, Greenland, Iceland, the Faroes, Great Britain, Ireland, Norway, Sweden, Finland, Switzerland, W. Germany, Austria, the Soviet Union and Japan.

16. UNITED KINGDOM

No information this year. An account was given for the 1981 report and verbal information was given at the meeting in 1983.

17. USA

An account of studies on genetics of natural and stocked populations together with aquaculture genetics, was presented as a separate report to the Mariculture Committee in 1983 (F:11, compiled by Arlene Longwell).

18. USSR

No information.

FISH AND CRAYFISH BROOD STOCKS MAINTAINED
BY THE FINNISH GAME AND FISHERIES RESEARCH INSTITUTE,
FISHERIES DIVISION

IN 1984

Compiled by Irma Kallio

Explanations of the abbreviations of the names of the fish culture stations

EVO IFARS: The Evo Inland Fisheries and Aquaculture Research Station
16970 EVO

INARI FCS: The Inari Fish Culture Station
99870 INARI

KÄYLÄ FCS: The Käylä Fish Culture Station
93850 KÄYLÄ

LFCRS: The Laukaa Fish Culture Research Station
41360 VALKOLA

MUONIO FCS: The Särkijärvi Fish Culture Station
99300 MUONIO

PORLA FCS: The Porla Fish Culture Station
08100 LOHJA

CFCSNF: The Central Fish Culture Station for Northern Finland
A 658 Ohtaoja
91999 OULU

SARMIJÄRVI FCS: The Sarmijärvi Fish Culture Station (owned by
The National Board of Waters.
Managed by the Fisheries Division)
A 780 kalanviljelylaitos
99800 IVALO

SIMUNANKOSKI FCS: The Simunankoski Fish Culture Station
41480 SIMUNANKOSKI

SUOVU FCS: The Suovu Fish Culture Station
Suovu PJ
71999 KUOPIO

CFCSEF: The Central Fish Culture Station for Eastern Finland
58175 ENONKOSKI

Fish and crayfish brood stocks maintained by the Finnish Game and Fisheries Research Institute, Fisheries Division in 1984. (Abbreviations brood stock type - D=domestic; M=mixed domestic and natural brood stocks; N=natural reproducing "wild" brood stock. Use - FL=fingerling stocking in lakes; FS=fingerling stocking in streams; PTL=put-and-take stocking in lakes; PTS=put-and-take stocking in streams; OS=other special uses).

Atlantic salmon, Salmo salar

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
ML 6/73	Autumn	235	M	River Tornionjoki	PTS	natural stock
ML 8/74	"	290	M	" "	PTS	" "
CFCSNF						
ML 2/67	"	200	D	" Iijoki	PTS	1. hatchery generation
ML 1/71	"	100	D	" "	PTS	1. " "
ML 1/72	"	185	D	" "	PTS	2. " "
ML 2-6/74	"	320	D	" "	PTS	2. " "
ML 7/75	"	100	D	" "	PTS	2. " "
ML 1, 3/76	"	900	D	" "	PTS	2. " "
ML 1, 3/77		1 950	D			
CFCSNF						
ML 1, 3/77	Autumn	2 500	D	River Iijoki	PTS	2. hatchery generation
ML 1, 2/78	"	1 000	D	" "	PTS	2. " "
ML 1/78	"	800	D	" "	PTS	2. " "
LF CRS (1983)						
1972	"	168	D	" Neva	PTS	1. " "
1974	"	158	D	" "	PTS	1. " "
1976	"	343	D	" "	PTS	1. " "
1978	"	610	D	" "	PTS	1. " "
1979	"	541	D			
LF CRS						
LJ-82/221-222	"	15 000	D	" Iijoki	PTS	2. " "

Landlocked salmon 1984, Salmo salar m. sebago Girard,

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
JL 1/80	Autumn	20 000	D	River Pielisjoki	PTS	2. hatchery generation
JL 4/80	"	3 900	D	" "	PTS	1. " "
MUONIO FCS 1/77	"	264	D	" Vuoksi, Pielisjoki	PTS	2. " "
SUOVU FCS -77	"	600	D	" Pielisjoki	PTS	1. " "
LFCRS -75/K1	"	60	D	" Pielisjoki, Lieksanjoki	PTS	2. " "
-76/189L	"	141	D	" Pielisjoki	PTS	1. " "

Sea trout 1984, Salmo trutta (m. trutta) L.

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
MT 7/70	Autumn	76	D, M	River Iijoki	PTS	1 hatchery generation
MT 4/73	"	418	D, M	" "	PTS	2. " "
MT 3/77	"	300	D, M	" "	PTS	2. " "
MT 1/78	"	500	D, M	" "	PTS	1. " "
CFCSNF						
MT 9/77	"	678	D, M	" Lestijoki	PTS	2. " "
MT 5/78	"	500	D, M	" "	PTS	2. " "
MUONIO FCS						
MT 1-75	"	203	M	" Tornionjoki	PTS	1. " "
MT 1/81	"	500	M	" "	PTS	
PORLA FCS						
MT 77	"	320	M	" Isojoki	PTS	
LFCRS						
MT1-74/27	"	201	M	" "	PTS	2. " "
MT1-76/155L	"	275	M	" "	PTS	1. " "
MT1-79/	"	465	M	" "	PTS	2. " "
MTD-78/126	"	359	D	" Daljoki	PTS	2. " "
			M			

Brown trout 1984, *Salmo trutta m. lacustris*

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF JT 2/71	Autumn	60	D, M?	River Varisjoki	PTS	2. hatchery generation
CFCSNF JT 5/74	"	420	M	Rautalampi River System	PTS	2. " "
JT 2/78	"	1 000	M	Simunankoski	PTS	3. " "
SUOVU FCS -78	"	670	M	Rautalampi River System, Äyskoski	PTS	1. " "
SIMUNANKOSKI FCS -79	"	136	M	Rautalampi River System	PTS	2. " "
PORLA SFH -79	"	275	M	- " -	PTS	
LFCRS JTR -74/1, 9, 95	"	80	M	- " -	PTS	2. hatchery generation
JTR -75/A64	"	170	M	- " -	PTS	2. " "
JTR -80/142)	"	520	M	- " -	PTS	2. " "
JTR -80/140-142)	"		M	- " -	PTS	2. " "
LFCRS JTV -78/178 L	"	600	M	River Vuoksi	PTS	1. " "
MUONIO SFH 2/73		304	M	Lake Pallasjärvi	PTL, PTS	1. " "
MUONIO SFH 2/76		142	M	Lake Pöyrisjärvi	PTL PTS	1. " "

Brown trout 1984, *Salmo trutta m. lacustris* L.

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
2/73	Autumn	199	M	River Juutuanjoki	PTS	1. hatchery generation
21/75	"	602	M	" "	PTS	1. " "
INARI FCS						
21/75	"	750	M	" "	PTS	1. " "
21/78	"	200	M	" "	PTS	1. " "
21/79	"	2 000	M	" "	PTS	1. " "
SARMIJÄRVI FCS						
JT 21/75	"	754	M	" "	PTS	1. " "
JT 21/77	"	1 600	M	" "	PTS	1. " "
JT ss 81-83	"	35	M	River Ivaloĵoki	PTS	Natural stock
JT 1/77	"	760	M, D	" Kiellajoki	PTS	1. hatchery generation
CFCSNF						
1/69, 4/72, 1/73	"	13	M	" Kitkajoki	PTS	1. " "
1/77	"	995	M	" "	PTS	2. " "
KÄYLÄ FCS						
JT ss/76, 77	"	100	M	" "	PTS	Natural stock
1/77	"	2 000	M	" "	PTS	2. hatchery generation

Brown trout, Salmo trutta m. fario L.
non-migratory

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF PTss 79, 81, 82	Autumn	51	M	River Kemijoki	PTS	
PT 3/80	"	800	M	" "	PTS	
PT 1/71	"	138	M	River Iijoki,	PTS	
PT 1/74	"	185	M	Ohtaoja " "	PTS	
EVO IFARS PT 1/79	"	400	M	River Luutaoja	PTS	

Arctic char 1984, *Salvelinus alpinus*

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSEF SN ss 83	Autumn	18	M	Lake Saimaa	PTL	natural stock
CFCSNF IN 1/63	September-	7	M	Lake Inari	PTL	1. hatchery generation
IN 1/66	November	90	M	" "	PTL	1. " "
IN 1/70		130	M	" "	PTL	2. " "
Sarmijärvi FCS IN 3/74		200	M	" "	PTL	1. " "
IN 1, 2/80		2 200	M	" "	PTL	

Lake trout, *Salvelinus namaycush* (Walbaum)

CFCSNF HN 1/63	September	97	D	Lake Superior, USA	PTL	2. hatchery generation
HN 1/68		100		" "	PTL	2. " "
HN 2/71		107		" "	PTL	2. " "
HNnl/55		10	D	" "	PTL	1. " "
Sarmijärvi FCS HN 1/76		300	D	" "	PTL	2. " "
HN 3/76	September	300	D	" Opeongo	PTL	

Brook trout, *Salvelinus fontinalis* (Mitchell)

CFCSNF PN 1/81	September	650	M, D	Unknown	PTS	3. hatchery generation
PN 1/78	October	200	M, D	" , USA	PTS	3. " "

1984 Rainbow trout, *Salmo gairdneri* (Richardson)

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
1/80	Spring	178	D	Unknown		
1/81	"	1 000	D	"		
LFCRS						
KLA-80/170	Spring	400	D	American		Donaldson and Steelhead hybrid stock
KLA-13-77/129	approx.	100	D	A-13, American		
KLA-13-78/132	"	100	D	A-13		
KLD-77/207	approx.	150	D	Donaldson		
KLK-78/165	approx.	200	D	Kamloops		
KLK-80/168	"	400	D	"		
KLT-80/169	approx.	400	D	Danish		

Whitefish, Coregonus peled (Gmelin) s. Berg

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
<u>C. peled</u>						
CFCSNF	Sept.-December (Oct.-January)					
PeS, 1/76, ss 72		400	M	Unknown.		Roe imported from the Soviet Union in 1965
PeS 1/76		350	M	"		" " " "
LFCS				"		" " " "
SP 81/1	approx.	1 000	M			1. hatchery generation
SP-77		460	M			

Whitefish, Coregonus pidschian (Gmelin)

CFCSNF						
Pos 1/75		100	M	Lake Oivankijärvi		1. hatchery generation
INARI FCS						
Pos ss 78-80	approx.	50	M	" Ivalojoiki		natural stock
KÄYLÄ FCS						
Pos 1/75	"	200	M	" Oivankijärvi		1. hatchery generation
SARMIJÄRVI FCS						
Pos /79	"	300	M	River Ivalojoiki		1. " "

Whitefish, Coregonus muksun (Pallas)

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
CFCSNF						
P1 S 1/76	Autumn	720	M, N	River Nivanvirta	PTL, PTS	1. hatchery generation
P1 S 1/74		600			PTL, PTS	1. " "
P1 S 1/75		770			PTL, PTS	1. " "
P1 S 13/78		2 880		" Koitajoki	PTL, PTS	1. " "
P1 S 18/80		3 000		" "	PTL, PTS	1. " "
P1 S 1, 2/72		279		" Partakoski and Rauhanvirta	PTL, PTS	1. " "
P1 S 1/71		230			PTL, PTS	
P1 S ss 72-74		73	N		PTL, PTS	natural stock
LFCRS						
SM-74/1		259	M			1. hatchery generation
SM-75/1		659		" Koitajoki, Koitere		1. " "
SM-77/1		892		Lake Karhujärvi, Tervonsalmi		1. " "
SM-78/2		436				1. " "
SM-79/1		2 000		River Koitajoki, Koitere		1. " "
SIMUNANKOSKI FCS						
-78;		608				
SARMIJÄRVI FCS						
/79	approx.	800		Lake Joutsenjärvi		1. " "

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
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Grayling, Thymallus thymallus L.

CFCSNF						
H 1/72, ss 80	Spring	120	M	River Iijoki	PTS	Natural stock
H 1/79, 2/80	April- May	600	M	" "	PTS	1. hatchery generation
ss/83		87	M	River Iijoki	PTS	Natural stock
SIMUNANKOSKI FCS						
H	May	200	M	Rautalampi River System	PTS	Natural stock

Pike-perch, Stizostedion lucioperca L.

PORLA FCS	June					
Ku	Spring	30	M, N		PTL	1. hatchery generation
SIMUNANKOSKI FCS						
Ku		126	M, N		PTL	

Carp, Cyprinus carpio L.

SIMUNANKOSKI FCS	May- June					
Ka	JKP-77/1	150	D	Unknown	PTL	Imported from Sweden
PORLA FCS						
Ka		75	D	"	PTL	" " "

Fish hatchery and brood stock	Spawning season	Breeding adults	Broodstock type	Origin	Use	Other information
<u>Crayfish, <i>Astacus astacus</i></u>						
EVO IFARS	Autumn	1 800	M, N	Finnish		
<u>American Signal Crayfish, <i>Pacifastacus leniusculus</i> (Dana)</u>						
PORLA FCS	Autumn	370	M, N			

AQUACULTURE STRAINS IN NORWAY

RAINBOW TROUT The rainbow trout in use for fish farming in Norway originally came from Denmark, and there have been some import even in later years. Also from Finland some live rainbow trout are imported. At the moment it is rather difficult to distinguish between "strains" of rainbow trout in Norway, although certainly there are differences between the populations in different fish farms. Such differences almost certainly are derived from artificial or/and "seminatural" selection due to adaptation to the particular fish farming conditions. Mass selection for higher growth rate and late maturation have been carried out by some fish farmers, as well as selection within families has been carried out at research stations. However, exchange of material between fish farms and from research stations to fish farms still take place and tend to level out the differences that have been obtained. Tentatively, I prefer to speak about four strains of rainbow trout in Norway, although I am not sure whether they really fit into the definition of "strains" given by Kincaid (1981), and accepted by the Working Group of Genetics.

LOCATION	SPAWNING SEASON	CHARACTERISTICS AND USE
A. Institute of Aquaculture Research, Sunndalsøra and Averøya	Jan.-March	Intensive aquaculture, family- and individual selection for productive traits
B. Institute of Marine Research, Bergen	- " -	- " -
C. ErosLaks, Bjordal	- " -	Intensive aquaculture, selected for late maturation
D. Øksna Bruk, Sandnes (and others)	- " -	Intensive aquaculture, probably moderately changed since imported

ATLANTIC SALMON

The salmon in use for intensive aquaculture in Norway are derived from wild populations during the 70-ties. There are great variations between natural river populations also in productive traits, but the culture "strains" are synthesized from different natural populations, and it is difficult to distinguish between salmon strains with specific characteristics at the moment. Also in this species mass selection for better performance is carried out by the fish farmers as well as both mass selection and family selection are performed at research stations. A selection program in charge of the fish farmers associations has recently been accepted. Besides this seminatural selection for adaptation to fish farming condition (domestication) is supposed to take place continuously. Similar to the rainbow trout also "strains" of salmon are kept at the research station and at some hatcheries (private firms), for instance MOWI A/S, Bergen. However, it is still more difficult to characterize salmon strains than rainbow trout strains, and these "strains" evidently do not fulfil the definition. Most of the strains are also frequently mixed up in the fish farms, and they are not tested for productive performance under identical environmental conditions. Until better characterized it

therefore seems to be of minor interest to list such strains.

OYSTERS

Production of flat oyster seed take place at 8 selected small fjords ("poller") where the temperature is particular high compared to natural environment. At some of these sites broodstock from the same "poll" has been used for up to 25 generations, and thus specific characteristics may have been developed. Strain performance (growth and mortality rates) are now being tested on oysters from different "polls" as well as on oysters imported from Scotland.

OTHER SPECIES

In connection with research work and development of technique for farming of "new" species, research stations and private companies are building up broodstocks of halibut, scallops, Crassostrea gigas, lobsters, crayfish and others. At the moment such broodstocks cannot be listed as "strains", but if the main experiments are successful, development of separate culture strains of such species might well be of great significance.

APPENDIX 4

A NATIONAL TROUT STRAIN REGISTRY FOR SWEDEN

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Background

Effective management of fishery resources calls for, among other things, detailed information on strain performance under different conditions, be they in hatcheries or in the natural environment. Prerequisites to achieve these goals are e.g. the establishment of objective criteria for strain identification, the creation of a system of nomenclature to identify the strains and finally, the development of a standardized system for collecting material and disseminating the data resulting to user organizations. This system has multiple advantages. One is that it provides the potential buyer with a performance list of the strains and species he/she is interested in. As a consequence of that the buyer may also find out which strain is most suitable for the specific natural environment or continued use for food production. Lastly, the continuing loss of suitable trout habitat in lakes and rivers due to environment degradation calls for objective information on endangered stocks, i.e. the registry provides information facilitating the preservation of natural strains.

Already in 1974 the Fish and Wildlife Service of the United States evaluated performance characteristics of the most common rainbow trout strains in the country and the continuing task of refining the system as well as including other trout species was and is in the hands of Dr. Harold L. Kincaid of the Fish Genetics Station in Kearneysville, West Virginia. It should thus come as little surprise that the national strain registry to be currently initiated in Sweden heavily relies on the experience gathered in the US. The creation of the Experimental Fish Station at Kålarne, province of Jämtland (northern Sweden), in 1983 will serve as the focal point for evaluating a suitable set of traits to be used for characterizing the different strains of trout. Apart from this high priority task the Station will also engage in projects aiming at studying the genetic and ecological effects of hatchery environments on fish populations, in particular with reference to small brood stocks and untypical selection.

Current status of the Registry

In September 1984 the National Board of Fisheries completed a survey resulting in a report "The Conservation of the Genetic Resources of Swedish Fish Stocks". Despite the fact that only freshwater species were covered, and in detail only salmonids, this survey at least summarized the status of natural populations and brood stocks currently used in Atlantic salmon, sea-run and landlocked trout (*Salmo trutta*), Arctic char and grayling, i.e. all the native salmonids. The report concluded that all 34 populations of Atlantic

salmon should be considered endangered and that preservation measures should be considered for 74 populations of sea-run trout, 73 populations of landlocked trout, 51 of char and 19 of grayling.

The report included information on the following traits known for each population:

- 1) where the population comes from (lake/river and county)
- 2) whether it spawns in lakes or rivers (inlets/outlets)
- 3) whether it lives "-
- 4) whether the adults are small/large
- 5) specific traits other than those above (not necessarily proven hereditary)
- 6) local conditions/threats including an estimation of local, regional, national interest
- 7) classification according to "conservation value" - local/regional or national.

Unfortunately, only scanty information exists on actual population size (number of breeding adults) and data on spawning season and type of use is not included. Since only a few brood stocks are kept exclusively in hatcheries the problem of potential bottle-neck effects pertains to natural populations, with the exception of some rather inbred salmon stocks, without any effects of domestication. A very comprehensive survey of the biology and status of the Swedish salmon stocks was performed by a Government Committee in 1984. By strongly advocating measures to protect all salmon stocks in the country, they also proposed genetic studies to obtain better information on the possible genetic distinctness of the stocks of separate rivers, despite the long-practised use of transplanting fish from one river to another when too few breeding adults had returned to the native river. If the suggestions related to research materialize we will be able to compile a salmon strain registry, because all the major stocks are being artificially reared.

A standard environment and characteristics program is currently being tested at the Kälarne Station. This is designed to follow closely the one used by Kincaid (1981). Traits like percentage hatching, deformed fry, fingerling survival and growth will be given, but also whether fish of varying age tend to take food at/near the surface, in midwater or at the bottom. The latter trait may prove interesting to anglers. Also, frequencies of isozyme systems expressing genetic variability will be used to characterize strains. Catchability will be established by angling only, including data on frequency and duration. This standardized program will be tested first on Arctic char, then continuing on to landlocked brown trout and grayling, and possibly in the future followed by natural and domesticated strains of exotic rainbow trout and brook trout. It is envisaged that all data on these strains will be compiled in data base form for increased accessibility.

REPORT FOR GENETICS WORKING GROUP, MAY 1985 - NEW TECHNIQUES - DNA AND CHROMOSOMES

DNA, Molecular Cytogenetics, and Mobile Genetic Elements
in Relation to Genetic Variation, Stock Identification,
and to New Methods of Genetic Selection*

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and Implications for Genetic Selection

The proportion of singular, unique DNA sequences in various plant and animal chromosome complements varies from as low as 1-2% of all the DNA to half or more. Yet, most evidence indicates that it is these unique DNA sequences that contain most of the transcribed structural genes of the organism. The greatest portion of the DNA sequences in many eukaryotes consists of short, largely non-transcribed sequences repeated in long arrays (Setlow and Hollaender, 1983). This is called satellite DNA because in density ultracentrifugation of isolated DNA it forms such a distinctly different band. Repetitive DNA sequences fall into 2 categories, moderately repetitive and highly repetitive, simple sequence DNA. These can be further subdivided into related families by density gradient ultracentrifugation and biochemical reassociation kinetics. Moderately repetitive sequences can comprise about 5-30% of all the DNA. For several reasons, repetitive DNA is believed to have a regulatory function in transcription of unique, single sequence DNA coding for proteins. As such, it could be the element on which selection operates when this is for quantitative or commercial performance traits.

A 1975 study reported on the DNA sequence organization of the American oyster, Crassostrea virginica, and the surf clam, Spisula solidissima, among 3 other marine

* This is excerpted from a longer paper prepared for the Shellfish Seminar, La Rochelle, France, March 4-9, "Current understanding and technology of chromosomes, shellfish resources and culture."

invertebrates (Goldberg et al., 1975). All the genomes studied showed a major fraction of the unique sequence DNA to be interspersed with short repetitive sequences. Most of this DNA interspersed with coding unique sequence DNA is probably of the very short sequence type.

Total DNA content of the American oyster is near the low extreme in content for pelecypod mollusks, about one-half that of the surf clam, and only one-eighth that of the largest bivalve genome reported (Hinegardner, 1974). The genome of the oyster was calculated to contain 4.7×10^8 nucleotide pairs. At least 60% of these are non-repetitive unique sequences.

Another study (Kamalay et al., 1976) provided additional information on DNA sequence repetition in the genome of the American oyster. This oyster at least has 2 classes of moderately repetitive DNA. Of these two, the longer is repeated on the average of 20 times when and where it occurs, and comprises 28% of all the C. virginica DNA. The other class represents 10% of the DNA. The latter contains sequences repeated about 3000 times. In addition, at least 1% of the oyster DNA of the repetitive class has related, but not identical sequences.

The spectrum of repetitive DNA in the American oyster is similar to that found in other mollusks. However, unique sequence DNA in the surf clam was found to be longer, more complex than in the oyster. In Spisula, less DNA fell into the longer sequence DNA lengths of the repetitive class. Also, Spisula has a shorter sequence class with more repeat copies. Further, reiteration of DNA sequences is higher in the gastropods (Collier, 1971; Collier and Tucci, 1980) than in bivalves.

This general pattern of DNA sequence organization of our familiar oysters and clams is similar to that in Xenopus (a toad), and different from that in Drosophila.

Two developments have made it possible to locate types of DNA sequences along chromosomes. One is the in situ hybridization method developed by Gall and Pardue (1971). The other is the molecular cloning technique for isolating and purifying DNA sequences. Once a particular DNA sequence has been multiplied by cloning in a

suitable prokaryotic vector, it can be characterized in detail by restriction endonuclease analysis, or by complete nucleotide sequencing. Estimates can then be made of the copy number of the genes per chromosome set (Brandham and Bennett, 1983).

The in situ hybridization technique consists of making the chromosome spreads on microscope slides, denaturing the DNA, and preparing a single-stranded complementary DNA or RNA probe. The probe is then allowed to hybridize onto recognition sites along lengths of the exposed chromosomes on the slides (Macgregor and Varley, 1983).

This method can be used for quite precise localization of gene sequences. The technique could make selection for commercially desirable traits more efficient by detecting the presence of particular genomic DNA sequences or blocks of genes that determine character with savings in time, labor, and grow-out space (Flavell, 1981; National Research Council, 1982). (In the future monoclonal antibodies might similarly be used.) It has, perhaps more presently, obvious use in stock identification, and as a measure of genetic variability in natural populations. Such DNA diagnostic techniques are expected to be rather routinely used in breeding programs which will begin to have a dependency on them in the next 10 to 30 years.

Cloning of segments of DNA up to 40 Kb in suitable prokaryotes has become essentially routine in the last 5 years (Bennett et al., 1981; Dahl et al., 1981). At the University of California, Santa Barbara, D. E. Morse has recently started a collection of cloned DNA of the large, red, commercial abalone, Haliotis rufescens (Morse, 1984).

To date, best results have been obtained on in situ DNA chromosome hybridization techniques with the repetitive sequence DNA, and this class of DNA is easily isolated and purified. However, so many advances are being made in this area the state of the technology is bound to change rapidly. Until recently it has been necessary to use radioactively labelled probes and autoradiography to view the hybridization sites on the chromosomes. Now a technique has been developed in

which probes are instead labelled with a biotin derivative of cytidine. Probes are detected on the chromosomes with an immunological technique, histochemical or antibody sandwiching method (Langer and Ward, 1981). This procedure is easier to use, and it is just as sensitive as the radioactive label.

The in situ hybridization technique has already provided a fine resolution of repetitive DNA sequences along the chromosomes of some groups (Hamkaló and Papaconstantinov, 1973; Brutlag, 1980; Flavell, 1981; Setlow and Hollaender, 1983; Brandham and Bennett, 1983). The nucleolus-organizing regions of the chromosomes have been found to be synonymous with the longer tandem arrays of repetitive DNA sequences. These regions contain the genes coding for 18 and 28 S ribosomal RNA associated with the nucleolus. (Ribosomes are essential for protein synthesis.) Ribosomal gene clusters in the bivalves were projected to represent less than 5% of the genome (Collier, 1971; Kidder, 1976a, b). These genes are more redundant in higher organisms. In groups where the ribosomal gene clusters are distributed over a number of chromosomes there is remarkable inter-individual variation in the location, site, size and number of clusters. This occurs even within strains and inbred lines (Bennett et al., 1981; Brandham and Bennett, 1983). NOR bands of the chromosomes represent those ribosomal gene clusters which actively transcribed their message in the preceding cell cycle. There is a genomic control over transcription of these genes which has been much studied (McClintock, 1934; Crosby, 1957; Crosby Longwell and Svihla, 1960; Phillips, 1978; Flavell and O'Dell, 1979).

There seems to be a conservatism in the evolution of the nucleotide sequences of the ribosomal genes. Even ribosomal DNA from insects will hybridize with complementary ribosomal DNA from amphibians. This should enable us to use already cloned DNA of other mammals, Drosophila and sea urchins in initiating our studies on these nucleolar gene clusters in shellfish and in some salmonid studies.

Much of the more moderately repetitive DNA is clustered at the centromeres of the chromosomes or at their ends, but some is interspersed along the arm lengths. Its distribution corresponds to the C chromosome bands or heterochromatic chromosome segments. Moderately repetitive DNA is ubiquitous, but it is present in different forms, different lengths, different chromosome positions, and different amounts in various species and populations. This is just as are C bands.

In mammals, yeast and Drosophila most such dispersed, moderately repeated DNA sequences are movable genetic elements, or mobile genetic elements appear to be responsible for the transposition of these moderately repeated gene clusters from chromosome to chromosome within the cell (Pollard, 1984; Shapiro, 1983; Syvanen, 1984).

Mobile Genetic Elements - Genetic Variation in Populations and Domestication

Movable genetic elements were first recognized by Barbara McClintock more than 3 decades ago in maize through their genetic effects which were inconsistent with accepted models of mutation (see McClintock, 1957). Apparent movement of these elements about the maize chromosomes affected the regulation and expression of a variety of genetic markers. Changes they induced were inherited. The mobile genetic elements of corn came to be recognized as similar to the transposable elements of prokaryotes. Such phenomena are known now to be quite common in eukaryotes (Shapiro, 1983; Pollard, 1984; Syvanen, 1984).

Advantage has been taken of the large salivary gland chromosomes in Drosophila and of the in situ hybridization technique to study chromosome position of the dispersed, repetitive DNA that comprises mobile genetic elements. Its location was found to be highly variable between species and between stocks, and also between individuals, and to change in time over laboratory culture (Young, 1979; Engels, 1983).

As well as causing changes in gene regulation and expression, these motile elements can cause large-scale chromosome rearrangements of the sort already described here. The effects of these rearrangements range all the way from altered expression of single genes to subversion of the entire genome of the organism altering its overall genetic structure and information (Engels, 1983). Importantly, these alterations often appear to be in physiological response to the organism's environment. Mobile elements are responsible for the chromosome rearrangements that accompany formation of active antibody-producing cells in mammals, and they are responsible for the switch in mating type in yeast. Many mobile elements though produce no clear phenotypic change. Their only effect seems to be to increase mutability.

At times, mobile genetic elements may be responsible for a majority of the observed mutation in a population, and they account for the high mutation frequency occurring in various populations from time to time. It is interesting to speculate here that the large numbers of fissions and fusions of the centromeres of fish chromosomes which provide polymorphisms even at the level of cells within individuals, and between individuals, and between populations may be under the influence of mobile genetic elements.

Motile elements are also involved in hybrid dysgenesis, the phenomenon of increased mutation, long observed in hybrid zones in nature, and in forced, artificially produced hybrid crosses. Hybrid dysgenesis is considered as having served an important role in the cultivation process of our crops and domestication of animals. Many of the mutations arising in various dysgenic hybrid crosses are unstable just as are those induced by known motile genetic elements. We can only wonder what role mobile genetic elements might play in development of shellfish culture, what role they have always played in resource populations of shellfish, and if we here are to learn anything about their function in our molluscan resources.

The vast array of quasi-stable genetic elements now known to occur widely is difficult to incorporate into the traditional view of evolution as slow accumulation of genetic change and speciation over long times. By disrupting gene regulatory systems, these elements seem capable of effecting change over shorter periods than ever anticipated. This could provide a mechanism for speciation, and account for the rapid genetic changes which must have occurred in domestication of animals.

Particular significances of mobile elements for quantitative genetics, population genetics, traditional plant, animal, aquaculture breeding and domestication of aquaculture species have barely begun to be considered, or not considered at all as yet. The powerful effect of selective breeding probably does not produce progress so much by recombination and selection of structural genes as by recombination and selection of gene regulatory sequences of the chromosomes. If so, selective breeding might be more efficiently, directly, practically practiced in the future on regulatory sequences than on particular production traits. Regulatory genes, the mobile elements, might be practically modified using recombinant and other DNA techniques. Clearly, however, for now there are mobile genetic elements that can and do alter gene regulatory systems. It is ignorance of gene regulatory systems that limits utility of gene transfers recombinant DNA technology makes possible. For such reasons, competitive grants in molecular biology available from the U.S. Department of Agriculture for work on plant, animal or aquaculture species of food value are being prioritized on the basis - not of promise of immediate practical applications which will always have restricted use - but rather on the basis of how much might be learned of genome structure and function of the particular commercial species (Committee on Biotechnology, Division of Agriculture, 1983, 1984).

Because moderately repetitive DNA and the C chromosome bands have been linked to mobile genetic elements, their potential for instability should be kept in mind

in using nucleotide sequences, size, and chromosome position of these gene clusters or chromosome bands in any such population studies of shellfish we might hope to see in the future. Because these non-coding gene clusters further may be implicated in cell differentiation, it seems wise and interesting to compare patterns from different tissue sources.

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