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Growth And Developmental Stability Of Inbred, Outbred And Inter-strain Hybrid Tilapia (*Oreochromis Niloticus*) In Two Diet Regimes

by Nancy Shackell

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Abstract

Aquaculture genetic improvement programs may be ineffective if the sole objective is to increase growth rate. Stress resistance is equally important but is difficult to measure. In this study, developmental stability was used to characterize dietary stress resistance of 2 strains of tilapia (Oreochromis niloticus). Developmental stability was measured as the inverse of (1) asymmetry of bilateral traits, e.g. paired fins (Asymmetry index) and (2) the amount of scale circulus deformation (Circulus Deformation index). Growth and developmental stability of inbred, outbred and inter-strain hybrid lines of tilapia were studied in 2 dietary regimes. Growth rate was significantly faster in the constant high protein regime (CE) than in the fluctuating low-high-low protein regime (FE). Hybrid progeny of the "Nifi" and "Israel" strains grew the fastest, followed by the Israel strain and lastly the Nifi strain.

As measured by the Asymmetry index, developmental stability was unexpectedly higher in regime FE than in regime CE; partially because the data was dependent on size. In contrast, developmental stability was higher in regime CE using the Circulus Deformation index. The Asymmetry index showed that (1) the inbred Nifi line had the lowest developmental stability and that (2) the Nifi strain had lower developmental stability than the Israel and hybrid strains. The Circulus Deformation index showed that (1) inbred lines had lower developmental stability than outbred lines, (2) the Nifi strain had lower developmental stability than the Israel strain and that (3) the hybrid progeny had higher developmental stability than their mid-parent value. The Asymmetry index is difficult to measure and was found to distinguish poorly between diets and among lines. Its use is therefore better suited to the controlled environments of laboratories than to the variable environments on farms. The Circulus Deformation index is more practical, and was found to be a more discriminative index of developmental stability. It has potential application as (1) an indicator of environmental and/or genetic stress and as (2) a selection criterion, in conjunction with growth, in genetic improvement programs.

List of Symbols

Abbreviation	Meaning
Experimental:	
regime FE	Fluctuating low protein-high protein-low protein diet
regime CE	Constant high protein diet
Nifi, Israel	Two tilapia strains maintained at Binangonan, R.P.
Lines (A-F)	A-F refer to the genetic composition and strain of origin.
	A (Nifi) and C (Israel) are inbred progeny of brother-sister mating, B (Nifi) and D (Israel) are progeny of unrelated parents, E (Israel X Nifi) and F (Nifi X Israel) are hybrid progeny of two strains; 4 families (4 separate matings in each line A-F). In summary, there were 4 families/ 6 lines/ 2 regimes combinations representing 48 tanks (40 fish per tank) (40 fish X 48 tanks=1920 fish in total)
Strains	Nifi, Israel, Hybrid (Israel X Nifi, Nifi X Israel)
Cross	Inbred (progeny of brother sister mating), Outbred (progeny of unrelated Nifi or Israel parents), Hybrid (progeny of Israel female X Nifi male parents or progeny of Nifi female X Israel male parents).
Growth intervals:	
growth 2:	growth from week 0 to week 2
growth 4:	growth from week 2 to week 4
growth 6:	growth from week 4 to week 6
growth ENT:	growth from week 0 to week 6 (a measure of growth for the whole experiment, where Sampling Time=6 and Sampling Time-1=0.
Body Morphology:	

FA	Fluctuating asymmetry
Characters(1-4)	Morphological characters used in FA analysis
Asymmetry 1	Estimate of bilateral asymmetry using a variance component, statisically isolated from other sources of variation.
Asymmetry 2	Variance of individual siyned difference between left and right.

Statistical:

ANOVA	Analysis of Variance
MS	Mean Square
DF	Degrees of Freedom
P	Probability of significance

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Chapter 1. Introduction

1 General Introduction

Genetic improvement programs in aquaculture are not yet a research priority and have a short history relative to programs in agriculture and animal husbandry (Pullin and Capili 1988; Lannan et al. 1989). A major problem is that it is difficult to detect the genetic variance component of fish growth, because growth is easily modified by environmental variation (Kinghorn 1983; Uraiwan and Doyle 1986; Tave, 1988; Allendorf 1988). Genetic variation does exist on an evolutionary scale. In fact, fish have a capacity for rapid speciation, as is exemplified by the family Cichlidae. Meyer (1990a) estimates that there are more than 500 endemic cichlid species in each of the 3 largest African lakes. Many of these species exhibit trophic polymorphism, which attests to the ability of the cichlidae to evolve (Meyer 1987, 1990b; Witte et al 1990). Clearly, practical techniques are required to measure accurately genetic merit in variable ecosystems. A research objective at the Binangonan Freshwater Station, Philippines, is to develop techniques to assess genetic potential of tilapia (Oreochromis niloticus) broodstocks. At the station, my

objective was to study growth and developmental stability of inbred, outbred and inter-strain hybrid tilapia broods under two different diet regimes.

In this introduction, I start by explaining the rationale of the thesis. I present arguments to validate the economic importance of developmental stability as an indicator of stress resistance. Then I provide a short theoretical background on developmental stability and phenotypic plasticity. Many researchers have used population levels of morphological variation as an index of either developmental stability or phenotypic plasticity, yet the conceptual differences between them result in opposite interpretations of the same data. Two alternative methods of measuring developmental stability are then defined and a general methodology is presented. The chapter ends with an outline of the thesis structure.

2 Thesis Rationale

Increased growth rate is usually the primary objective during the initial stage of genetic improvement programs (Jinks and Pooni 1988). However, experience with agricultural programs have shown that stress and disease resistance become equal in importance to growth as genetic

gains are achieved (Plucknett and Smith 1982, 1986). It is common that success in selecting primarily for high yield is done at the expense of stress resistance. Perhaps the best documented example comes from what is referred to as the "Green Revolution". In the Philippine rice industry, the International Rice Research Institute of Los Banos, Philippines, selected primarily for high yield varieties but they did not select for stability of yield across a range of environments. Rice varieties were selected under laboratory conditions for high yield of their reproductive parts at the cost of vegetative structure. Energy allocation was thus directed towards producing fruit and away from health maintenance. The resultant varieties were less competitive in regards to disease, to other plants and to other forms of stress (Parsons 1990a). In essence, the researchers discovered that the selected high yield varieties were less stable, i.e. they succumbed more readily to diseases and pests. These varieties require a monoculture system, with minimal environmental variation, which can only be achieved by expensive maintenance management, such as higher amounts of chemical fertilizers and pesticides (Plucknett and Smith 1986).

In other agro-industries, a positive correlation between yield and sensitivity was observed in Nicotiana rustica and N. tabacum. A notable observation was that some gene loci responsible for sensitivity, were different from

those responsible for yield (Jinks and Pooni 1988). Another example is drawn from a silviculture industry on the Northwest Pacific coast. The selection of faster growing trees has led to weaker wood as a result of an increase in the spacing between growth rings. The wood poses no problems for the pulpwood market but is not ideal for any construction requiring structural strength (Senft et al 1985 c.f. Maser 1990).

It appears that selection for a single commercial trait can result in an overall decrease in crop quality. As the field of crop genetics and animal husbandry progressed, biologists began to acknowledge the importance of overall stress resistance, not simply resistance to specific diseases, by selecting for growth stability across a range of environments (statistical methods in: Eberhart and Russell 1966; Hanson 1970; Westcott 1987; Wolff and Van Delden 1987; Witcombe 1988; Ariyo 1990). By way of a successful example, a wheat strain (Triticum aestivum) was developed for resistance to wheat leaf rust; unfortunately, it was also hypersensitive to environmental perturbations. Researchers are now concentrating efforts to develop a more stable strain with partial resistance, and report that the test strain is stable across a range of environments (Broers and Parleviet 1989). The notion is that systems in which selection is based solely on one commercial trait are often self-limiting (Schnell 1988).

In aquaculture, genetic improvement programs are in an early stage and superior growth is almost always the principal objective. Using the initial experience of agriculturists, fish breeders should consider selecting fast growing fish that are also stress resistant (Chevassus and Dorson 1990). Although they must adapt to domestication, fish will initially be resistant to environmental stress because of their inherent suite of defense mechanisms after 400 million years or so of natural selection. The importance of foreseeing future problems is especially great in developing countries, where fish farms tend to be variable and poorly controlled relative to terrestrial monoculture systems.

The geneticist's objective may then be to develop fish that are capable of good growth in poorly understood, poorly controlled and fluctuating ecosystems. The criterion of "merit" in the selection program thus incorporates both growth and stress resistance, and the question becomes how to define and measure stress resistance. Disease resistant fish can be considered of superior merit, but a commonly used indicator of superior disease resistance is survival. As the number of surviving individuals increases, the efficiency of selection decreases Chevassus and Dorson (1990). Chevassus and Dorson (1990) point out that

aquaculture geneticists are in dire need of indirect criteria for evaluating disease resistance "leading to individual characterization of healthy animals".

In this thesis, I use developmental stability, as measured by body and scale morphology, to characterize the stress resistance of individual tilapia. Merit (growth and developmental stability), as such, varies among individuals even in the same environment, because each individual reacts to environmental stress differently. I operationally define response to stress as a decrease in merit, that is, as a reduction in growth and developmental stability.

3 Background of Developmental Stability

Developmental Stability and Phenotypic Plasticity

Developmental stability (the ability to regulate development in order to produce one phenotype) and phenotypic plasticity (the ability to develop a range of phenotypes dependent on the local environment) are different developmental processes. Both processes can be defined as adaptive if they shape optimal phenotypes. Developmental stability refers to the regulatory action within a developmental pathway. In a stable pathway, phenotypic variation will be low despite environmental or genetic

perturbations. In less robust systems, we observe variation in response to perturbation. Phenotypic plasticity refers to the ability to follow several alternate developmental routes resulting in more than one phenotype. Therefore, adapted developmental pathways can be 1) stable and not plastic (developmental stability) and 2) stable and plastic (phenotypic plasticity). Both types are stable but the latter results in phenotypic polymorphism and a higher phenotypic variation. In essence, stability of developmental pathways means that the internal system can biochemically compensate for environmentally induced perturbations by either regulating one pathway (developmental stability) or by switching pathways (phenotypic plasticity) (Smith-Gill 1983; Lively 1986).

Students of life history refer to developmental stability and phenotypic plasticity as optimal life history strategies, if either is positively associated with fitness. (Fitness herein is broadly defined as anything that enhances survival, reproductive capacity or reproductive opportunities). The observed life history strategy is dependent on the variability of the environment, the trait, the trait's correlation with fitness and the evolutionary history of the organism (Orzack 1985; Strauss 1987; Falconer 1989). The assumptions underlying the relationship between each developmental process and fitness and the methods of measuring developmental stability and phenotypic plasticity

have led to considerable confusion in the literature. An increase in morphological variability has been used to indicate a breakdown in developmental stability, but from a different point of view, an increased morphological variance could indicate an adaptive phenotypically plastic response.

Differentiating between developmental stability and phenotypic plasticity

Earlier studies referred to developmental stability as developmental homeostasis and were largely influenced by Lerner (1954), Waddington (1959) and Thoday (1956). These people assumed that the ability to canalize a trait was adaptive, that is, the greater the ability to buffer a trait from environmental fluctuations, the higher the fitness. Although Lerner (1954) did not overtly proclaim homeostasis as a universal theory, his excellent work dominated subsequent theory on developmental stability. Later research acknowledged that homeostasis represents only one possible life history strategy.

A single canalized phenotype may be disadvantageous for organisms in which growth is characterized by a continuous (but not constant) increase in size, such as in plants and fish. Unlike warm-blooded organisms, the final size of many cold-blooded organisms is largely determined by the environment. Cold-blooded organisms are more responsive to environmental conditions. Developmental stability might

detract from the ability to respond favorably to variable environments (Orzack 1985; Strauss 1987). In organisms in which development is easily modified by the environment and which inhabit environments that are variable over generations, the optimal strategy is to have the ability to develop into a range of phenotypes; in essence, to exhibit phenotypic plasticity (Bradshaw 1965; Levin 1970; Schlichting 1986).

As fully explained further in the text, I use the variability of bilateral (bilateral asymmetry) and repeated (scale circulus deformations) structures on the same individual, to measure developmental stability. Morphological variability between individuals has been used as a measure of both developmental stability and phenotypic plasticity, which has led to some confusion in the literature (Schlichting 1986). Results have been interpreted assuming that either developmental stability or phenotypic plasticity is adaptive (enhancing fitness). If developmental stability is assumed to be adaptive, then morphological variability will be low. There is assumed to be one optimal phenotype and if developmental physiology is sufficiently regulated, organisms in a given population will be phenotypically similar at a given life stage. Within the population, stabilizing selection will cause more fit individuals to be in the central range of a frequency distribution, while less fit individuals will be on the

extremes. In contrast, if phenotypic plasticity is assumed to be adaptive, then morphological variability will be high as each individual in a population will develop into a phenotype well adapted to its own local micro-environment. Relatively fitter individuals will take advantage of a variable environment and maximize fitness under favorable conditions (Strauss 1987). Within the population, the relatively more fit individuals will not nessecarily be in the central range, instead they will be distributed in accordance with the frequency distribution of fitness.

Morphological variation and Genetic Composition

Heterozygosity, as measured by gel electrophoresis or by level of inbreeding, has been positively associated with fitness components (e.g. growth, developmental stability and reproductive output) and hence, has been used as an indirect measure of fitness (review in Mitton and Grant 1984; Allendorf and Leary 1986;). The mechanism of heterozygote superiority has not been completely resolved (Mitton and Grant 1984; Allendorf and Leary 1986; Zouros and Foltz, 1987). Four of the classical hypotheses of heterozygote superiority include 1) dominant alleles in a heterozygous state masking deleterious recessive alleles 2) heterozygosity increasing the physiological range of gene products (Koehn 1970 c.f Angus and Schultz 1983; empirical results reviewed in Mitton and Grant 1984 and in Zouros and

Foltz 1987) 3) overdominant alleles rendering the heterozygote more fit than either homozygote (Falconer 1989) 4) adapted gene combinations in which some loci are heterozygous. In any event, more closely related animals have a higher probability of combining similar alleles and creating more homozygous zygotes than do distantly related animals, therefore inbred population are more homozygous and empirical observations show that inbreeding can be deleterious (Falconer 1989).

As heterozygosity relates to morphological variation, Lerner (1954) proposed that heterozygosity confers a superior, therefore adaptive, canalization. If so, it follows that less heterozygous individuals will show decreased developmental stability. Again, if developmental stability is measured by morphological variance, the relationship between heterozygosity and morphological variability may not reflect superior developmental processes. The same reasoning as outlined previously in this text implies that increased morphological variance can result from several causes. Both increased and decreased morphological variation has been associated with the level of heterozygosity. For example, decreased morphological variance associated with heterozygotes has been observed in Fundulus heteroclitus (Mitton 1978) and Donaus plexipus (Eanes 1978) in side blotched lizards (Uta stansburiana) (Soule et al 1973), in herring (Clupea harengus L.) (King

1985), in Drosophila (Gupta 1978). McAndrew et al (1982) found no relationship between enzyme heterozygosity and the variance of three meristic traits in the flounder Pleuronectes platessa. Hanford (1980) found no association between heterozygosity and morphological variability in the rufous-collared sparrow (Zonotrichia capensis). The subject is reviewed in Livshits and Kobyliansky (1985) in Allendorf and Leary (1986) and in Zouros and Foltz (1987).

4 Thesis Methods of measuring Developmental Stability

The research on morphological variation as a measure of developmental stability is valuable, yet there is a serious drawback as it assumes that decreased morphological variance reflects a genetically superior developmental process. Two alternative measures of developmental stability are used in this thesis. I studied the developmental stability of tilapia by (1) measuring the magnitude of asymmetry of bilateral traits (more asymmetric fish have a lower developmental stability) and by (2) quantifying the abnormal development of the scale (highly deformed scales reflect a lower developmental stability).

The first method is called "Fluctuating Asymmetry" (FA) and has been advocated as an indicator of genetic and environmental stress (Jones 1987; Leary and Allendorf 1989;

Parsons 1990b). FA is a measure of the repeatability of development between right and left sides of bilateral organism. It assumes that the same genome is responsible for the development of the right and left hand sides and that bilateral symmetry is produced by coordination between genetically determined developmental processes. The magnitude of the difference between sides is a measure of a genotype's sensitivity to the environment, therefore genotypes with less buffering will be more asymmetric.

The second measure of developmental stability is the amount of deformation of scale circuli. The similarity among the repeated developmental event of circulus (calcified growth ridges) deposition on scales is a measure of developmental stability because the same genes express themselves again and again. Any deviation is because the gene products were somehow affected by the environment. Therefore, a higher number of deformed circuli on the scales reflect decreased developmental stability.

Neither measure of developmental stability is dependent on the level of within population morphological variation, that is, level of morphological variation is not assumed to be adaptive or non-adaptive. Instead, both measures assume an adaptive coordination between repeated, genetically determined developmental pathways. FA and circulus deformation, as measures of developmental stability, are further described in chapters 3 and 4, respectively.

5 General Materials and Methods

The following materials and methods apply for the whole thesis, specific materials and methods are contained in the relevant chapters.

Genetic Composition of Lines

The tilapia used in this study were created from 2 strains, namely Nifi and Israel (Z. Basiao, SEAFDEC,Rizal, Philippines) maintained at Binangonan Freshwater station in the Philippines. The experiments took place at the station. Preliminary experiments, to refine sampling techniques, were conducted on first generation (G1) populations of the Nifi and Israel strains. The major experiment was conducted over a period of ten weeks in 1989. Fish scales, sampled at the end of the experiment, were measured over a period of 9 months.

Breeding sets of one male and 3 females were placed in circular fibreglass tanks and fed commercial food pellet (appendix 1) ad libitum. When one female had produced a brood, the male and females were replaced into the mass population. Only 1 full-sib family was used from each set of

male/ female matings. The inbreeding coefficient F describes the degree of relationship among the parents relative to a base population of zero (Falconer 1989).

The full mating design produced 24 full-sib families:

A) Four Nifi families from 4 separate brother-sister matings. (Inbreeding coefficient F=.25).

B) Four Nifi families from 4 separate sets of unrelated parents (Inbreeding coefficient F=0).

C) Four Israeli families from 4 separate brother-sister matings. (Inbreeding coefficient F=.25).

D) Four Israeli families from 4 separate sets of unrelated parents (Inbreeding coefficient F=0).

E) Four families from 4 separate sets of Israeli mothers and Nifi fathers (Inbreeding coefficient F=0).

F) Four families from 4 separate sets of Nifi mothers and Israeli fathers (Inbreeding coefficient F=0).

The codes A-F refer to the level of inbreeding and strain of origin throughout the text.

Experimental methodology

One hundred fry were randomly chosen from each family at approximately 4 weeks of age. Fifty fish were allotted to the fluctuating environment (FE) group and 50 to the constant environment (CE) group. The total nutrient composition of both diets is listed in Appendix 1. Each group (48 groups of 50 fish each) was placed in a 250 litre

aerated polyethylene tank of standing water. Feces and debris were removed and one third of the water was changed daily in the tanks. The experimental run lasted 6 weeks. The FE groups were fed rice bran (low protein, 6.61%) from week 0 to week 2, commercial food pellet (high protein, 26%) from week 2 to week 4 and rice bran from week 4 to week 6. The CE groups were fed commercial food pellet throughou the experiment. Protein is not the only nutrient that differs between diets (see appendix 1). It is, however, the limiting factor in tilapia growth (Bowen 1982).

At the end of the experiment, 40 fish were sacrificed from each diet/line/family combination (4 families in 6 lines at 2 diet levels). The following traits were measured: standard length, weight, and right and left distances from 1) the posterior edge of eye socket to the most posterior edge of opercular bone, 2) the anterior insertion of pelvic fin to the tip of the first hard ray 3) the ventral insertion of the pectoral fin to the dorsal insertion of the pelvic fin and 4) the lower lateral line to the dorsal insertion of the pectoral fin. Scales were sampled 1 row above the upper lateral line and 3 rows back from the head and preserved in 10% buffered formalin.

Throughout the thesis, inbred and hybrid line values (growth and developmental stability) are compared to outbred lines using the following equations (Kincaid 1976a,b):

Inbred relative to Outbred:

((inbred line mean-outbred line mean)/outbred line mean)*100

Hybrid relative to Outbred to check for Heterosis (defined as when hybrid progeny yield higher values than the midparent value):

((hybrid line mean-midparent outbred line mean)/midparent outbred line mean)*100

Where midparent outbred line mean is the average of outbred Nifi and outbred Israel. Positive or negative values represent the percent increase or decrease (respectively) in value of either inbred or hybrid lines as compared to outbred lines.

6 Thesis Structure

The objective of the thesis was to examine the effect of dietary regime and inbreeding on growth and developmental stability. Developmental stability was measured by two methods, asymmetry of body morphology and abnormal scale morphology.

Chapter 1. Introduction: statement of thesis problem, concept definition and general experimental methodology.

Chapter 2. Growth: patterns of growth of inbred, outbred and hybrid lines under two different diet regimes.

Chapter 3. Developmental Stability of Body Morphology: the effect of regime and line on developmental stability as measured by asymmetry of body morphology. The relationship between asymmetry and growth is presented.

Chapter 4. Developmental Stability of Scale Morphology: the effect of regime and line on developmental stability and individual variability of growth, as measured by scale morphology. A composite index of genetic merit is constructed based on the relationship between abnormal scale measures and growth.

Chapter 5: The results are discussed, using evolutionary theory to evaluate the potential use of developmental stability as a tool in genetic improvement programs.

Chapter 2. Growth

1 Abstract

Growth of inbred, outbred and interstrain hybrid tilapia was compared under 2 dietary regimes. Fish in the fluctuating low-high-low protein regime (FE) grew more slowly than fish in the constant high protein regime (CE). The variance of growth was greatest during the first interval, after which, the mean and variance of growth both decreased. The difference in growth rates between regimes was not as pronounced in the interval in which the fish in regime FE were switched from rice bran to commercial food pellets. There was a significant difference among strains during all but the last interval; hybrids grew the fastest, followed by the Israeli strain and lastly the Nifi strain. There was a significant difference among the types of cross (inbred, outbred or hybrid) in the first interval; hybrids grew the fastest, followed by outbreds and lastly inbreds. Overall, inbred Nifi grew 11.52 % faster than outbred Nifi. Inbred Israel grew 17.37% slower than outbred Israel. Interstrain hybrid IXN (Israel X Nifi) grew 5.16% better than the midparent value while hybrid NXI grew 7.98 % better than the mid-parent value. At the level of strain, outbred Nifi grew 26.53% slower than outbred Israel. There were no significant interactions between regime and any genetic

composition factor (line, strain or cross). The correlation of family growth rates between the first and second interval was positive within regime, that between the first and third intervals was positive when regimes were pooled and within regime FE. Thus, families that grew relatively fast in the first two weeks continued to do so throughout the experiment.

2 Introduction

This chapter describes the general trends in growth. The major argument of my thesis is that growth is of great importance in genetic improvement programs but does not incorporate the equally important trait of stress resistance. The thesis objective was to develop indices of developmental stability as indicators of dietary stress resistance. Consequently, the chapter is quite short as my main interest in growth was its relationship to developmental stability (Chapters 3 and 4) and how growth and developmental stability might be used in a composite index of merit (Chapter 4).

Caged Nile tilapia survive well on natural food abundances (Hepher and Pruginin 1982). Supplemental feeding is commonly used in areas deficient in natural food populations or to enhance production. Protein is a limiting factor in the tilapia diet (Bowen 1982). The growth of Nile tilapia is relatively suppressed in fish fed 20% protein (Wee and Tuan 1988). Rice bran, used in the present study, is about 6.61% protein and suppresses both tilapia (R.Romana, unpubl.data) and milkfish (Chanos chanos) growth (Santiago et al 1989). I used rice bran to induce stress in order to observe the response of growth and developmental stability of tilapia.

Inbreeding and loss of genetic variation can have deleterious effects on fitness traits (reviewed in Wright 1977, Falconer 1989). Inbreeding in fish has had variable effects on growth. In some instances, growth is depressed in inbred fish (Kincaid 1976 a,b) while in others, inbreeding has had only minimal effects (Bondari and Dunham 1987; Gjerde 1988). The consensus is that the effect of inbreeding depends on the genetic history of the population and the number of families sampled. Some fish breeders purposely interbreed strains, in order to guard against the loss of genetic variation. In fact, hybridization is considered a potential tool for use in aquaculture (Ayles and Baker 1983). However, Gjerde (1988) contends that the maintenance of highly inbred lines in order to produce hybrid strains is too costly due to the poor viability of inbred lines and the large facilities required to maintain them. In this chapter, the growth of inbred, outbred and inter-strain hybrid tilapia in 2 different dietary regimes is compared.

3 Methods

Standard length and weight were measured at 0, 2, 4 and 6 weeks. Family mean standard lengths were used to estimate family mean growth rates. I consider standard length a more accurate measure of fish size because there is a higher

measurement error associated with sampling wet weights of small fish than with sampling lengths. The amount of water on each fish varies (despite attempts to control it) and thus biases weight estimates.

Fish in regime FE were fed rice bran for the first two weeks, commercial food pellet from week 2 to week 4, and then rice bran from week 4 to week 6. Fish in regime CE were fed commercial food pellet throughout. Family mean specific growth /day was measured as

$$SG = ((X_T - X_{T-1})/\text{no.of days})*100$$

Where

SG=specific growth; X_T=Family mean log standard length (mm) at time T; X_{T-1}=Family mean log standard length (mm) at time-1; no. of days=number of days

Family mean specific growth estimates (% log mm/day) were used as variables in ANOVA models to analyse the effect of regime and genetic composition (line, strain and cross) at each growth interval and for growth over the entire experiment. Growth intervals are referred to as follows:

growth 2: growth from week 0 to week 2

growth 4: growth from week 2 to week 4

growth 6: growth from week 4 to week 6

growth ENT: growth from week 0 to week 6 (a measure of growth for the ENTire experiment, where T=6 and T-1=0)

Statistical Analyses

Separate ANOVAs were used to determine the effect of line, strain and cross. Line refers to level of inbreeding and strain of origin (e.g.inbred Nifi), strain refers to Nifi, Israel or Hybrids, cross refers to inbred, outbred or hybrid (see List of Symbols). In the present study, the between family variation was high. This was not due to differences among tanks because each family was separated into 2 tanks, representing regime FE and CE. To estimate the effect of between family variation, families were nested within line in the ANOVAs used to detect regime and line effects. Because I wanted to present a simple overview of the general effects of strain and cross, families were not nested in the strain and cross ANOVAs.

Regime and Line

Four variables, growth 2,4,6 and ENT were each analysed by ANOVA with 2 factors, regime and line (families nested within line).

$$Y_{ik(j)}=\mu + R_i + L_j + F_{k(j)} + E_{ik(j)}$$

Regime, strain and cross

Strain and Cross effects were analysed in simple 2-way ANOVAs.

$$Y_{ijk}=\mu + R_i + Strain_j + E_{ijk}$$
$$Y_{ijk}=\mu + R_i + Cross_j + E_{ijk}$$

Where $Y_{ik(j)}$ is the mean of the k^{th} family nested within the j^{th} line in the i^{th} regime; μ is the population mean; R is the effect of the i^{th} regime (2 levels); L is the effect of the j^{th} line (6 levels); F is the effect of the k^{th} family (4 families) nested within the j^{th} line and E is the random error term.

Y_{ijk} is the mean of the j^{th} strain in the i^{th} regime; $Strain_j$ is the effect of jth strain (3 levels); $Cross_j$ is the effect of jth cross (3 levels). Interaction terms were included in the analyses to check for dissimilar response of lines, strains or crosses to level of regime.

4 Homogeneity of Variances Between Diets

Homogeneity of variance between regimes for each growth interval was analysed using Bartlett's test for homogeneity of group variances (Table 2.1). Variances were homogeneous

for all growth intervals. Note that mean growth was greater in regime CE during growth 2 and 6 yet greater in regime FE during growth 4 (Fig. 2.1). Fish within regime FE grew faster during growth 4, corresponding to when their regime was changed from rice bran to commercial pellet.

TABLE 2.1. Regime means and (+/-) standard deviations in parentheses (S.D.) of growth 2,4,6 and ENT: N=24 families in each regime.

Growth Interval	2	4	6	ENT
REGIME FE				
Mean	0.780	0.944	0.187	0.647
(S.D.)	(0.155)	(0.156)	(0.121)	(0.122)
REGIME CE				
Mean	1.316	0.673	0.423	0.812
(S.D.)	(0.211)	(0.122)	(0.147)	(0.126)
[1]Chi-square	2.101	1.340	0.860	0.032
Probability	0.147	0.247	0.354	0.858

[1]Bartlett's Test for Homogeneity of Variances with 1 df. Probability denotes probability that group variances are significantly different.

5 ANOVA of Growth Intervals

In all the following analyses, there were no significant regime*genetic composition (line, strain, cross) effects, indicating that responses of lines, strains or crosses were similar to level of regime.

Regime and Line

Growth 2,4,6 and ENT were each analysed in separate ANOVAs with 2 factors, regime and line, and families nested within line. Line means at each growth interval are presented in Table 2.2. In each of the 4 analyses, the effect of regime and families nested within line were highly significant. The effect of line was insignificant in all

analyses (Fig 2.2, Table 2.3). However, the effect of line approached significance in growth 2, and in growth ENT ((F=2.52, F=2.083 respectively; $F_{critical}$= 2.77,df=5,18).

In each of the above analyses, I tested for differences between lines by a series of pairwise contrasts to see whether a significant difference between biologically meaningful pairs might be obscured in an ANOVA of 6 lines. The objective of pairwise contrasts was to compare 2 lines or strains, exclude the effect of other lines while holding the error rate constant. The contrasts, chosen a priori on biological grounds, were (1) inbred versus outbred (2) inbred versus hybrid (3) outbred versus hybrid (4) Nifi strain versus Israel strain. There was a significant pairwise contrast between pooled inbreds and pooled hybrids during growth 2 (F=8.69, $p<0.01$; $F_{critical}$=8.28, df=1,18). Hybrids grew faster than inbreds. There was a significant pairwise contrast between Nifi strain and Israel strain during growth 2 and growth ENT. The Israel strain grew faster than the Nifi strain.

Regime is clearly the major factor accounting for variation in growth in all 4 analyses. The F-ratio of the effect of regime was greatest during growth 2, followed by growth 4 and finally growth 6. The reduction of the F-ratio is due to the overall decrease over time (Fig 2.1). However, the mean growth was greater in regime FE during interval

growth 4, corresponding to when the fish switched from rice bran to commercial food pellet. The regime FE group responded to the change by increasing growth rate.

Strain Effect

To determine the effect of strain, growth 2,4,6 and ENT were analysed in ANOVA with 2 factors, regime and strain. The effect of regime was strongly significant. Strains were significantly different in all growth intervals except growth 6 (Table 2.4, Fig. 2.3). Overall, Hybrids grew fastest, followed by Israel and then Nifi.

Cross Effect

Regime has the same effect as in previous analyses. Crosses are significantly different only in growth 2 (Table 2.5, Fig 2.4). Overall, hybrids grew fastest, followed by outbreds and then inbreds.

Decrease of variance of growth over time

The difference between regimes and among lines, strains or crosses decreases over time, corresponding to a decrease in the mean and variance of growth. The variances of growth were significantly different among intervals (Table 2.6)

Comparisons among lines, strains and crosses

In order of decreasing value of growth ENT, outbred Israel> hybrid NXI > hybrid IXN > inbred Israel > inbred Nifi > outbred Nifi (Fig 2.2, Table 2.2). The percent increase or decrease of inbred and hybrid lines, calculated as the inbred or hybrid mean minus the outbred mean divided by outbred mean, is a measure of the effect of (1) inbreeding by one generation of brother-sister matings and of (2) interstrain hybrid matings. With respect to the Nifi strain, clearly no inbreeding depression is evident. The Nifi strain grew relatively very poorly. In fact, inbred Nifi grew 11.52 % faster than outbred Nifi. Inbred Israel grew 17.37% slower than outbred Israel. Hybrid IXN grew 5.16% better than the midparent value (calculated as the average of outbred Nifi and Israel) while hybrid NXI grew 7.98 % better than the mid-parent value. At the level of strain, outbred Nifi grew 26.53% slower than outbred Israel.

Given that the Nifi strain grew more poorly and that the effect of one generation of brother-sister mating is in the opposite direction of what was expected, perhaps the Nifi population, used in this experiment, is already fairly inbred.

TABLE 2.2. Line mean growth (%log mm/day) (pooled regimes) and (+/-) standard deviation in parentheses: N=8 family means /line, A=inbred Nifi, B=outbred Nifi, C=inbred Israel, D= outbred Israel, E= hybrid I X N, F= hybrid N X I.

Growth Interval	2	4	6	ENT
Line				
A	0.971	0.752	0.318	0.687
	(0.289)	(0.230)	(0.213)	(0.169)
B	0.860	0.724	0.267	0.616
	(0.286)	(0.157)	(0.179)	(0.138)
C	1.006	0.798	0.234	0.690
	(0.318)	(0.184)	(0.215)	(0.126)
D	1.164	0.899	0.375	0.835
	(0.382)	(0.239)	(0.128)	(0.142)
E	1.160	0.834	0.291	0.763
	(0.292)	(0.183)	(0.176)	(0.122)
F	1.127	0.844	0.343	0.784
	(0.366)	(0.171)	(0.171)	(0.115)

TABLE 2.3. Regime and Line Effect: Summary of ANOVA results for Growth 2, 4, 6 and ENT : FwL=family nested within line, DF=degrees of freedom, MS=mean square, P=probability of significance.

		Growth 2 R^2=0.976			Growth 4 R^2=0.940		
	DF	MS	F	P	MS	F	P
Regime	1	3.443	669.424	***	0.879	189.777	***
Line	5	0.121	2.520	ns	0.033	0.942	ns
FwL	18	0.048	9.298	***	0.035	7.562	***
Error	23	0.005			0.005		

		Growth 6 R^2=0.896			Growth ENT R^2=0.896		
	DF	MS	F	P	MS	F	P
Regime	1	0.669	98.617	***	0.328	255.951	***
Line	5	0.021	0.656	ns	0.050	2.083	ns
FwL	18	0.032	4.718	ns	0.024	18.393	***
Error	23	0.007			0.001		

ns= not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 2.4. Regime and Strain Effect: Summary of ANOVA results for Growth 2, 4, 6 and ENT : DF=degrees of freedom, MS=mean square, P=probability of significance.

		Growth 2 R^2=0.775			Growth 4 R^2=0.561		
	DF	MS	F	P	MS	F	P
Regime	1	3.443	133.876	***	0.879	49.473	***
Strain	2	0.225	8.757	**	0.060	3.370	*
Error	44	0.026			0.018		
		Growth 6 R^2=0.447			**Growth ENT** R^2=0.458		
	DF	MS	F	P	MS	F	P
Regime	1	0.669	35.341	***	0.328	25.785	***
Strain	2	0.002	0.127	ns	0.073	5.723	**
Error	44	0.019			0.013		

ns= not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 2.5. Regime and Cross Effect: Summary of ANOVA results for Growth 2, 4, 6 and ENT : DF=degrees of freedom, MS=mean square, P=probability of significance.

		Growth 2 R^2=0.730			Growth 4 R^2=0.512		
	DF	MS	F	P	MS	F	P
Regime	1	3.443	111.578	***	0.879	44.514	***
Cross	2	0.112	3.634	*	0.016	0.826	ns
Error	44	0.031			0.020		
		Growth 6 R^2=0.457			**Growth ENT** R^2=0.373		
	DF	MS	F	P	MS	F	P
Regime	1	0.669	35.987	***	0.328	22.291	***
Cross	2	0.010	0.531	ns	0.029	1.967	ns
Error	44	0.019			0.015		

ns= not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 2.6. Variability of Growth Intervals: Interval means and standard deviations (48 families in each interval) of growth 2,4,6 and ENT. (+/-) standard deviation, in parentheses).

Growth Interval	Mean	(+/- S.D.)
2	1.048	(0.327)
4	0.809	(0.195)
6	0.305	(0.179)
[1]Chi-square	21.219	
Probability	0.000	

[1]Bartlett's Test for Homogeneity of Group Variances with 1 df. Probability denotes probability that group variances are significantly different.

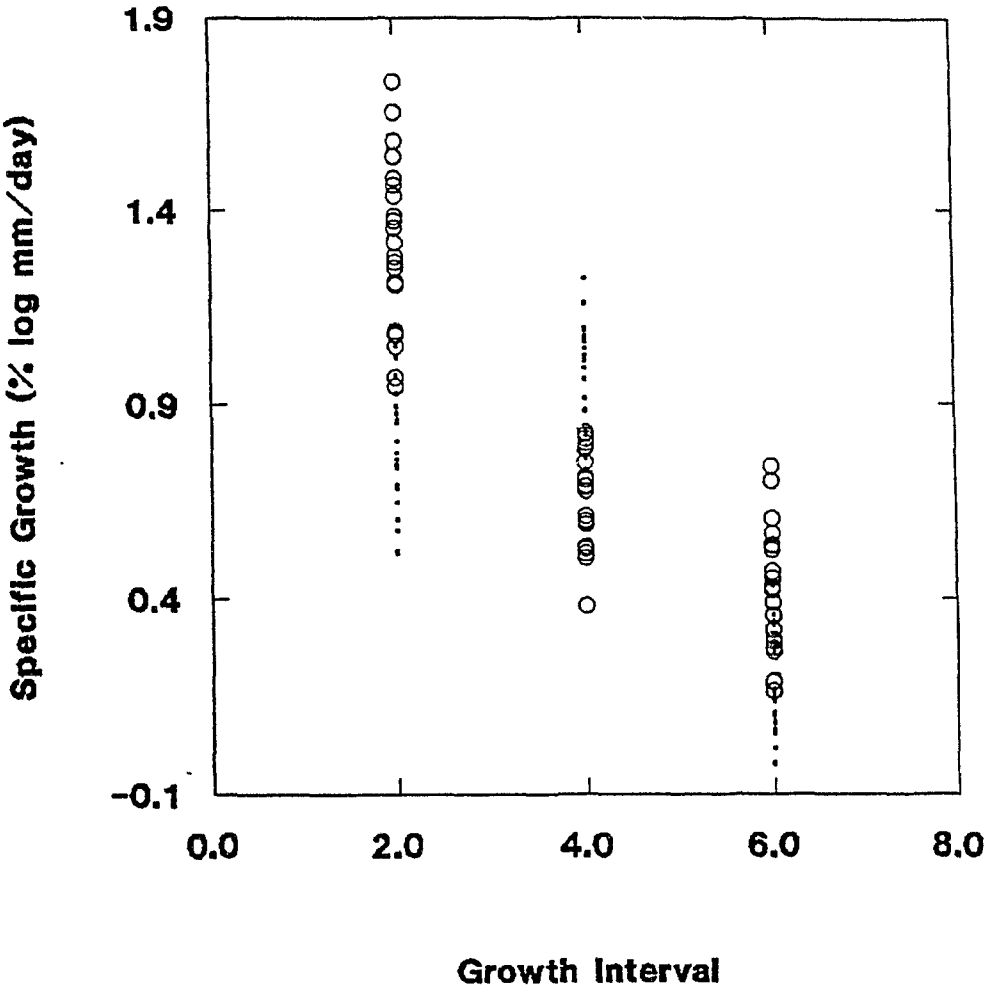


Fig. 2.1. Growth (% log mm/day) at Growth Intervals, 2,4 and 6. Filled circles= regime FE, Open circles= regime CE.

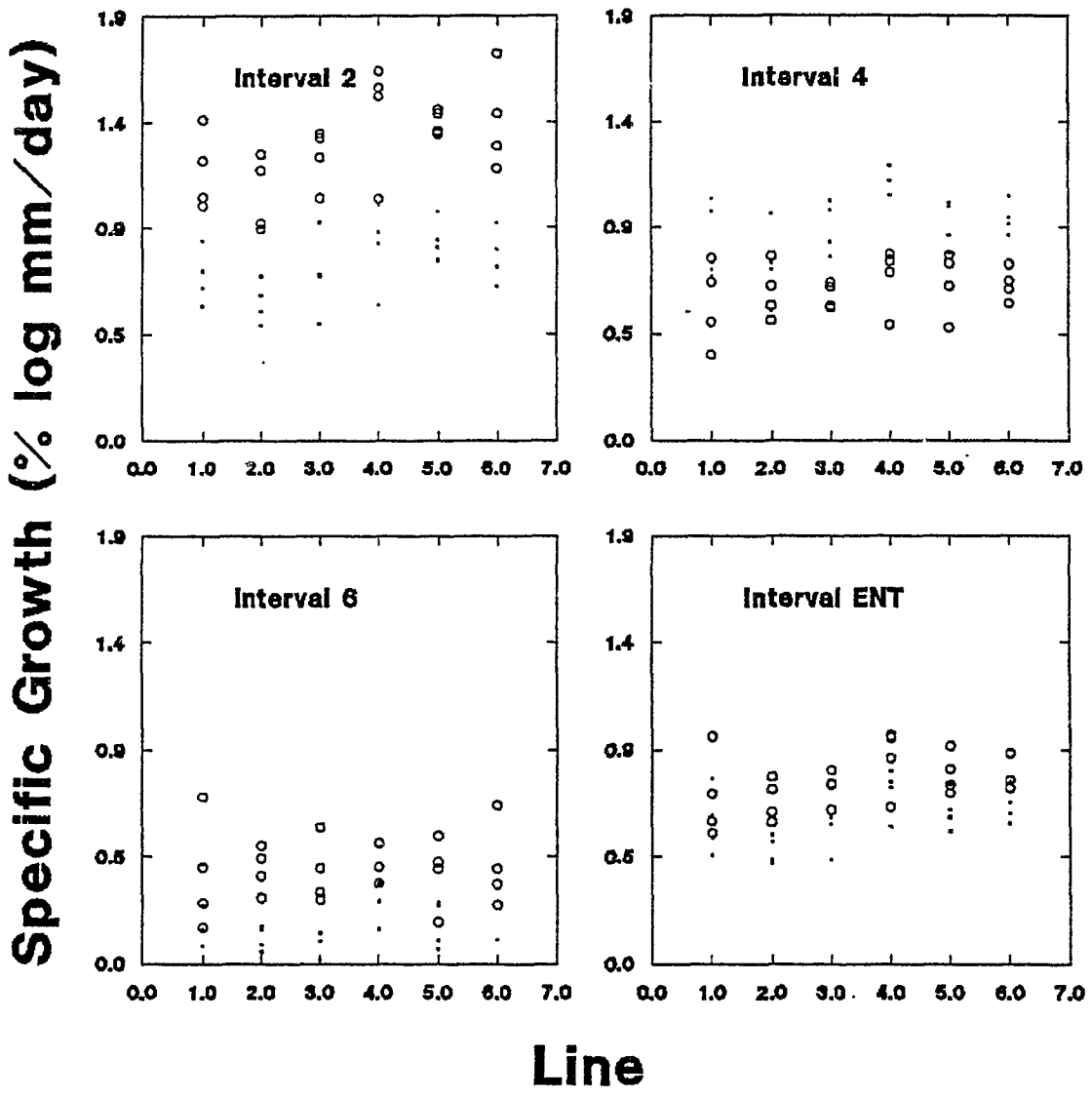


Fig. 2.2 Growth (% log mm/day) of Lines at each Interval 2,4 and 6 and of Interval ENT (estimate of growth for the whole period). Line codes: 1=inbred Nifi, 2=outbred Israel, 3=inbred Nifi, 4=outbred Israel, 5=Hybrid IXN, 6=Hybrid NXI; Filled circles= regime FE, Open circles= regime CE.

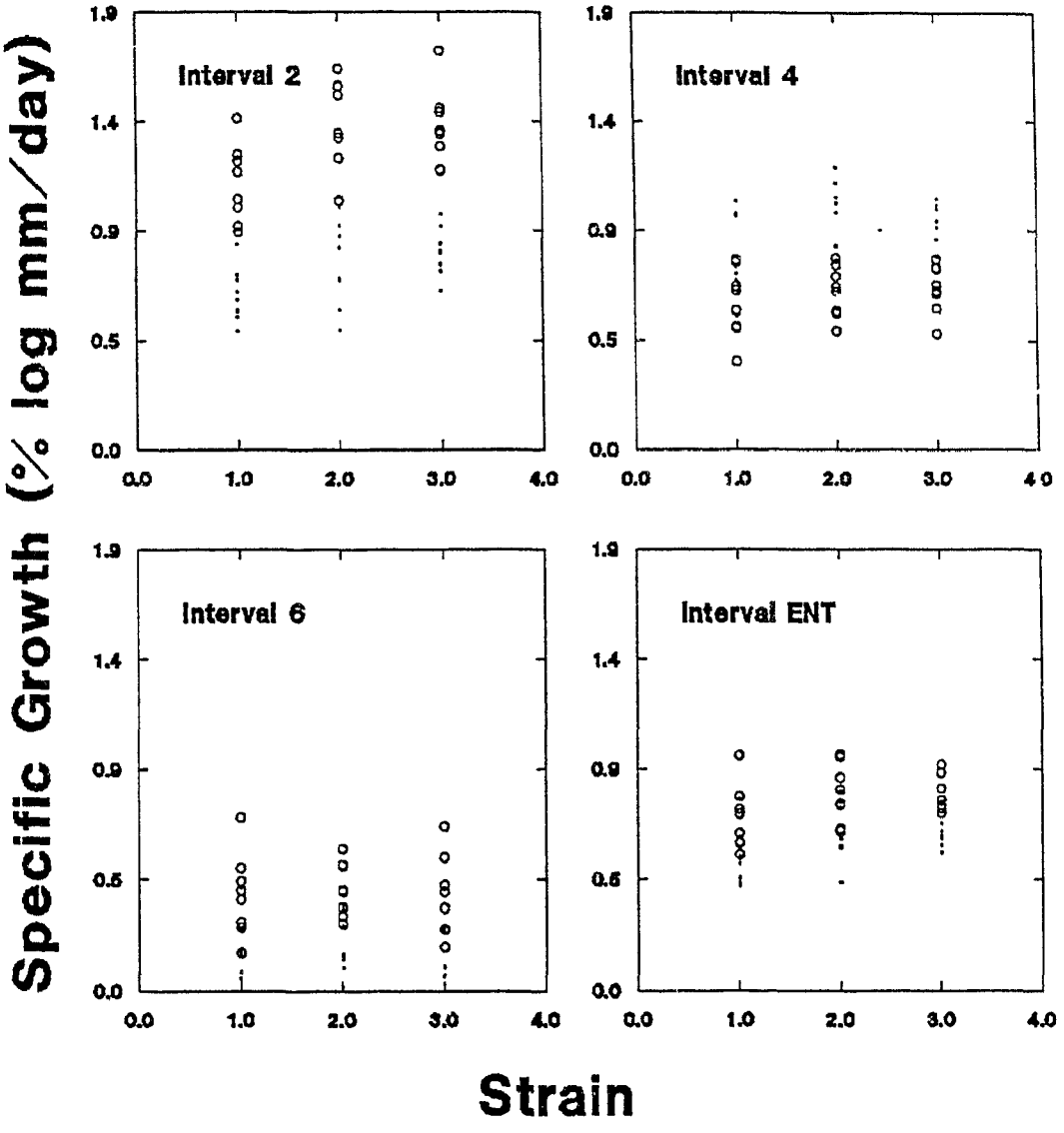


Fig. 2.3 Growth (% log mm/day) of Strains at each Interval 2,4 and 6 and of Interval ENT (estimate of growth for the whole period). Strain codes: 1=Nifi, 2=Israel, 3=Hybrids; Filled circles= regime FE, Open circles= regime CE.

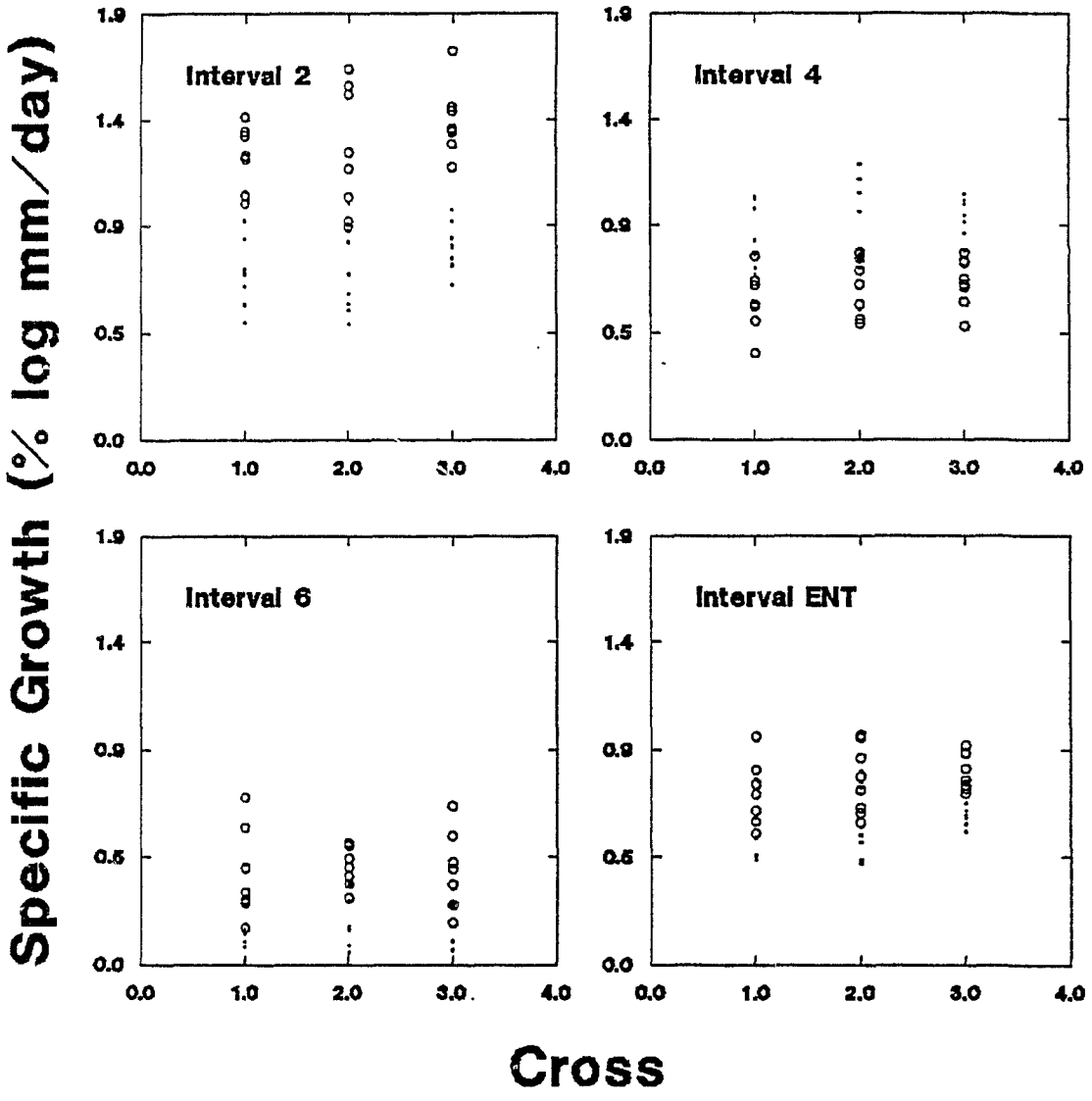


Fig. 2.4 Growth (% log mm/day) of Crosses at each Interval 2,4 and 6 and of Interval ENT (estimate of growth for the whole period). Cross codes: 1=Inbred, 2=Outbred, 3=Hybrids; Filled circles= regime FE, Open circles= regime CE.

6 Correlation among Growth Intervals 2,4,6

Family values were used to calculate correlation among growth intervals. The serial growth intervals were negatively correlated, when regimes are pooled, reflecting the change in regime of the rice bran group. The first and third interval were significantly positively correlated, meaning that populations which grew relatively fast during the first interval, also grew faster during the third interval (Table 2.7). Within regimes, the first and second intervals were positively correlated and the first and third interval was positive correlated within regime FE. The serial correlations decreased as the experiment progressed, possibly due to the decrease in both mean and variance of growth over the course of the experiment. However, families that grew relatively faster in the first two weeks continued to do so throughout the experiment.

TABLE 2.7. Pearson Product-moment correlation matrix of Growth Intervals 2, 4, 6 (N=48, df=46).

	REGIMES POOLED	
	Growth 2	Growth 4
Growth 4	-0.337*	
Growth 6	0.686***	-0.353*
	REGIME FE	
Growth 4	0.691***	
Growth 6	0.438*	0.231
	REGIME CE	
Growth 4	0.584***	
Growth 6	0.250	0.216

* Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level.

7 Discussion

Regime and Genetic Composition

Fish growth was suppressed in regime FE, as expected. The effect of regime is most evident during the first growth interval and least evident between weeks 2 and 4, when fish in regime FE were fed high protein commercial food pellets. The rank order of lines for overall growth is as follows: outbred Israel> hybrid NXI > hybrid IXN > inbred Israel > inbred Nifi > outbred Nifi. Outbred Nifi was not expected to rank last, indicating that either the number of family samples was too low or that the Nifi strain is suffering from a loss of genetic variation.

Genotype*Environment interaction

Genotype*environment interaction is a statistical term commonly used to describe the similarity of response of different genotypes to level of environment. An absence of genotype*environment interaction occurs when all genotypes respond equally across a range of environments. A simple example is that the performance of all genotypes in environment A is twice as good in environment B, that is, the genotypic values are additive. If the genotype responses to environment B are dissimilar (nonadditive) it means that performance in environment B cannot be predicted from that in environment A. That is, there is a presence of genotype*environment interaction. This is relevant to fish breeders who need to know if genotype performance is predictable across a range of environments. The importance of genotype*environment interactions is a contentious issue in the South East Asia aquaculture community. The scientists are currently deciding whether to produce an all-purpose tilapia strain or local specialized strains. Unfortunately, there is not enough data on the performance of available strains across a range of environments upon which to base their decision. I discuss this in detail in the final chapter but mention it here for context.

Genotype*environment interactions in growth have been observed in rainbow trout (Salmo gairdneri) (Klupp et al 1978; Ayles and Baker 1983; McKay et al 1984; Siitonen 1986;

Iwamoto et al 1986), in common carp (Cyprinus carpio) (Wohlfarth et al 1983) and in Nile tilapia (Uraiwan, S. unpubl. data). There were no significant genotype (line,strain,cross) * environment (regime) interactions in the analysis of growth in this study. The correlations of family growth values between the first and second intervals were significantly positive, corroborating the lack of genotype*environment interaction. However, two points are worth mentioning. Firstly, the effect of lines is marginally significant compared to the effect of regime, which may account for the lack of significant genotype*environment interaction. Secondly, Chapter 4 shows that genotype*environment interactions are important in the analysis of developmental stability. Consequently, the importance of genotype*environment interactions to genetic improvement programs should not be decided based on growth traits alone.

Chapter 3. Developmental Stability Of Body Morphology

1 Abstract

Developmental stability of inbred, outbred and hybrid tilapia in 2 dietary regimes was compared. Developmental stability was measured as the magnitude of fluctuating asymmetry (FA) of 4 morphological characters. Two FA methods were used: Method 1 (Asymmetry 1) is a variance component isolated from other (non-fluctuating) sources of asymmetry variation, using a statistical model of Palmer and Strobeck (1986). Method 2 (Asymmetry 2) is the variance of individual asymmetries (signed difference between left and right); a larger population variance indicates a higher level of asymmetry. Asymmetries of all characters were unexpectedly higher in the constant high protein regime (CE) than in the fluctuating low-high-low protein regime (FE) for both methods. The inbred "Nifi" line had the highest level of asymmetry. The "Nifi" strain had higher levels of asymmetry than the "Israel" and hybrid strains. Only one character was significantly negatively correlated with growth, and only within regime CE. On comparing the 2 methods, the analysis of Asymmetry 2 results in significant differences among factors not detected using Asymmetry 1. The lack of significance in Asymmetry 1 analyses stems from high variation between families within lines and low degrees

of freedom using family mean values. The Asymmetry 2 method uses individual instead of family mean values and is statistically more powerful. Given the practical and statistical rigor required to measure asymmetry and that the genetic and environmental differences were marginal, asymmetry is more suited to the controlled environment of the laboratory than to the variable conditions found on tilapia farms.

2 Introduction

This chapter describes developmental stability, as measured by the fluctuating asymmetry (FA) of 4 morphological characters. As was explained in Chapter 1, FA is presumed to result from variation in the repeated expression of genes during development. Below, I elaborate on the concept of FA and present empirical examples from the literature.

"Fluctuating" asymmetry is so called because it refers to the morphological asymmetry of an inherited bilateral trait caused by fluctuating internal and external environments during development (Lerner 1954). It is different from non-deleterious forms of asymmetry such as "directional" in which one side is always larger than the

"antiasymmetry", in which one side is consistently larger, but larger left and larger right sides occur with equal frequency in a population. FA differs in that it occurs randomly on either side of members of a population. The development of each member of the population is subject to potential disruption of a developmental process. Some individuals will be more asymmetric on the left and others, on the right. Palmer and Strobeck (1986) and Parsons (1990) provide thorough reviews of the FA literature.

(i) Asymmetry and Genes

FA has been negatively related to the level of heterozygosity, as measured by gel electrophoresis or level of inbreeding. Although results are not universally consistent, there are numerous convincing studies. A negative relationship between asymmetry and heterozygosity was found in side-blotched lizards (Uta stansburiana) (Soule et al 1973, Soule 1979), in salmonids (Leary et al 1984; Leary et al 1985a,b; Blanco et al 1990), in aquatic bivalves (Unionidae) (Kat 1982), in Drosophila melanogaster (Biemont 1983), in Poeciliopsis monacha (Vrijenhoek and Lerman 1982), in Apis mellifera (Bruckner 1976), in Tisbe holothuriae (Fava and Martini 1988) and in Drosophila melanogaster (Biemont 1983). However, Biemont and Terzian 1988 observed no difference in FA values between Drosophila lines selected for high and low viability.

Increased levels of asymmetry were found in the cheetah (Acinonyx jubotus) as compared to three other Felidae species (Wayne et al 1986) and in mice (Leamy 1984, Leamy and Atchley 1985, Leamy 1986) as a result of inbreeding. Atchley et al (1984) observed that mice with single deleterious autosomal recessive have higher asymmetry than the homozygous wildtype. And a final example, the reduced developmental stability of hatchery trout (Salmo clarki lewisi) was associated with an elevated frequency of morphological deformities (Leary et al 1985c).

Conversely, it has been widely observed that hybrid populations can have higher levels of asymmetry than do parental strains. This phenomenon has been reported in the hybrid progeny of two species of fireants (Solenopsis invicta and S. richteri) (Ross and Robertson (1990), in hybrid progeny of Enneacanthus gloriosus and E. obesus (Pisces) (Graham and Felley 1985) in hybrid progeny of pink (Oncorhynchus keta) and chum (O. gorbuscha) (Beacham and Withler 1987) and in 2 interstrain hybrids of rainbow trout (Salmo gairdneri) (Ferguson 1986). And, of course, there are studies that show no FA differences among hybrids and parentals. Lamb et al (1990) studied FA of hylid frogs (Hyla cinera and H. gratiosa). The interspecific hybrids did not have significantly different levels of FA from either parental strain. Asymmetry levels of Bluegill sunfish (Lepomis macrochirus) from an intergraded zone were

not significantly different from geographically distinct parental populations (Felley 1980). And finally, the results of a study on hybrid Poeciliopsis populations (Angus and Schultz 1983) are only weakly supportive of increased FA in hybrid populations.

The hypotheses to explain that hybridization may show decreased developmental stability is that if each parental genome has evolved separate adaptive combination of alleles (epistatic interaction) (Dobzhansky c.f. Felley 1980) then hybrid progeny will show decreased developmental stability as a result of incompatibility between parental genomes. In theory, as genetic distance between parental populations increases, there will be a point at which developmental stability breaks down as a result of divergent parental genomic combinations (Vrijenhoek and Lerman 1982).

(ii) Asymmetry and Environmental Stress

A prediction of Lerner's (1954) theory is that stress will decrease developmental stability, especially in less heterozygous animals. Several studies report that differences among genotypes are more easily detected in stressful environments. For example, the relationship between heterozygosity and growth rate in Ulinia lateralis is enhanced in stressful environments (Scott and Koehn 1988 c.f. Koehn 1989). Govindaraju and Dancik (1987) found no difference between the response of heterozygous and

homozygous strains of jackpines (Pinus banksiana) to different environments, but did find a relationship between growth and heterozygosity in a relatively highly stressed environment (see Govindaraju and Dancik 1987 for citations of further evidence that heterotic effects are only detected under stressful conditions). Doyle and Talbot 1988 and Shackell and Doyle (1989) observed that growth differences among individual tilapia (Oreochromus sp.) were more apparent during relatively slower growth periods. Overall, the evidence suggests that differential growth abilities are more apparent under stressful conditions.

Relatively stressful environments can cause an increase in FA. The results of studies on the relationship between stress and developmental stability are inconclusive insofar that it is difficult to define stress solely in environmental terms. Some studies report a positive relationship between asymmetry and environmental stress as in fish living in polluted areas (Valentine et al 1973), fish from a mercury contaminated pond (Ames et al 1979), muskrats (Ondatra zibethicus) from marginal habitats (Pankakoski 1985), Drosophila melanogaster raised at different temperatures (Parsons 1990b), Drosophila melanogaster raised in constant and fluctuating environments (Bradley 1980) and in the Fourhorn sculpin (Myoxocephalus quadriconis) after a one year experimental exposure to heavy metals (Bengtsson and Larson 1986). In

other studies, there has been no association between asymmetry and stress as in populations of Brook trout (Salvelinus fontinalis), White sucker (Catostomus commersoni)and Lake chub (Couesis plumbeus) in acidified and unacidified lakes (Jagoe and Haines 1985) or in Tisbe holothuriae raised at different salinities (Fava and Martini 1988) or in fish from heated and non-heated reservoirs (Ames et al 1979).

Clearly, stress must be defined both by a description of the environment and by the response of the organism to that environment. Stress must be severe enough to cause morphological effects in order to be detected by asymmetry measures. Some forms of stress will not affect the metabolic pathways which control development of morphological structures (Parsons 1990b). However,it does appear that asymmetry and stress can be positively associated if the stress is sufficiently severe. In this study, I used a protein deficient rice bran diet. Rice bran slows tilapia growth and causes circulus deformation (R. Romana, unpubl. data) and suppresses growth in milkfish (Chanos chanos), relative to other artificial or natural diets (Santiago et al. 1989).

Perhaps the most important influence on the interpretation of asymmetry variation is the measurement method. Palmer and Strobeck (1986) exhaustively reviewed analytical methods of measuring FA and conclude that the FA

signal can be small. Consequently, the accuracy of measuring must be very precise in order to detect true differences among FA samples. Despite the difficulties of detecting FA, there is empirical evidence on negative relationships between FA and heterozygosity, and between FA and stress resistance, in a variety of species.

As FA is only one of several sources of bilateral asymmetry my goal was to estimate FA while excluding other sources of asymmetric variation. Two analytical methods are used to compare bilateral asymmetry between regimes, among lines, strains and crosses. Method 1 (Asymmetry 1) is a variance component isolated from other (non-fluctuating) sources of asymmetry variation, using a statistical model of Palmer and Strobeck (1986). Method 2 (Asymmetry 2) is the variance of individual asymmetries (signed difference between left and right); a larger population variance indicates a higher level of asymmetry. Signed differences are tested for other sources of asymmetry, i.e. directional symmetry or antisymmetry. Asymmetry 1 and 2 values are compared between regimes, among lines among strains and among crosses.

3 RESULTS: Method 1

3.1 Statistical methods of estimating FA

Forty fish from each of 48 tanks were sampled. Duplicate measures of 4 morphological characters were measured on the left and right sides of each fish. In a preliminary study, I measured both morphological (continuous) and meristic (discrete, countable) traits. However, meristic traits are determined early in development, before I could impose a dietary stress. Also, the left and right sides of meristic traits frequently had identical counts and when they did not, the difference between sides was only 1 or 2 counts. Also, the measurement error often equalled or exceeded the difference. Jagoe and Haines (1985) report measurement errors of fish meristic characters to be 10-37% of total variation, but only 1-8% for morphological characters. In addition, the phenotypic variation of meristic traits is often discrete, yet the underlying genetic variation is assumed to be continuous (Swain 1987). Therefore, phenotypic variation of discrete meristic characters may not accurately reflect the underlying genetic variation and thus may bias estimates of asymmetry. In light of all this, I sampled only morphological traits. The 4 morphological measurements are shown in Fig. 3.1.

The difference between 2 sides of a bilateral organism can be slight, especially on small organisms, so that perceived differences may actually be due to mistakes in measurement. In this study, each fish was measured twice to reduce measurement error. Most left and right measures were within 2-3% character size of each other. I used a set of digital calipers to measure the traits, my helper read the calipers and asked me to repeat extremely dissimilar measurements of a trait until we were both satisfied that we had taken the most accurate measure. More than 2 measurements had to be taken on about 10 % of the fish. The data were used to estimate FA. Two methods were used, herein referred to as Asymmetry 1 and Asymmetry 2.

METHOD 1: (Asymmetry 1): The first method follows a design of Palmer and Strobeck (1986). The variance of the fluctuating differences is an estimate of asymmetry at the population (family/line/regime) level. However, asymmetry variation consists of several components, namely 1) directional asymmetry: variation due to one side being consistently larger than another (Van Valen 1962) 2) antisymmetry: variation due to either the left or right being larger and with no consistent bias towards one side or the other 3) size: asymmetry variation dependent on size or asymmetry that increases as size increases 4) measurement error: variation due to inaccurate sampling and 5)

fluctuating asymmetry: variation due to random differences between right and left sides, that is ascribed to mistakes during development (Van Valen 1962), hereafter referred to as FA. The FA component was isolated from total variation for each of 48 populations, by using the following ANOVA model of Palmer and Strobeck (1986). Note that antisymmetry is included in the FA estimate:

$$Y_{ijk} = u + S_i + I_j + SI_{ij} + e_{ijk}$$

where Y_{ijk}= right or left side of an individual; S_i=effect of ith side (i=2); I_j=effect of jth individual (j=40); SI_{ij}= effect of interaction between sides and individual; e_{ijk}= effect of kth measure in subgroup ij. The expected mean squares are presented in Table 3.1.

The variation components are interpreted as follows:

Between SIDES: Directional asymmetry or, variation due to morphological measure being consistently greater on one side.

Among INDIVIDUALS: Variation dependent on size.

Interaction of SIDES* INDIVIDUALS: The interaction term represents non-additive variation. It is the remaining variation above and beyond all other sources, that is, FA and antisymmetry. In the absence of antisymmetry, the interaction term is an estimate of FA of a population.
ERROR: Variation due to differences between duplicate measurements of sides on each fish.

The interaction term was isolated by subtracting the error term from the mean square interaction term and dividing by the number of measurements. For each of 4 morphological character, 48 Asymmetry 1 values were estimated from 48 anova models. Average Asymmetry 1 was calculated as the log (average of characters (1-4)). Variance components are typically chi-squared distributed, therefore Asymmetry 1 values were multiplied by 100 (scaling factor) and log-transformed prior to subsequent parametric analyses (modified method of Leamy 1984). Then, to test differences among populations, Asymmetry 1 values were used in a 2 factor ANOVA, regime and line, with families nested within lines.

METHOD 2: (Asymmetry 2) The average of duplicate right and left measures for each individual were computed and individual asymmetries were calculated as the average right minus the average left. The population variance of signed

differences is an estimate of FA. A relatively high population variance indicates that there are more highly asymmetric individuals in that population. Asymmetry 2 values were used 1) to analyse frequency distributions of individual asymmetries and 2) to analyse homogeneity of variances of signed differences between regimes, among lines, among strains and among crosses.

In summary, Asymmetry 1 is an estimate of population asymmetry, isolated from variation due to directional asymmetry, difference among size of individuals and measurement error. Asymmetry 2 estimates individual asymmetry, the population level of asymmetry is measured as the population variance of individual asymmetries.

TABLE 3.1. Expected mean squares of ANOVA model used to estimate FA: Ind=individual SXI=Sides*Individual interaction[1].

Source	df	Expected Mean Squares	Interpretation
Sides	(S-1)	$\sigma^2_m+m\sigma^2_i+m(J/S-1\Sigma\alpha^2)$	Directional Asymmetry
Ind	(J-1)	$\sigma^2_m+m\sigma^2_i+ms\sigma^2_j$	Size
SXI	(S-1)(J-1)	$\sigma^2_m+m\sigma^2_i$	FA/2 plus antisymmetry
Error	SJ(M-1)	$\sigma 2_m$	Measurement

[1]Adapted from Palmer and Strobeck (1986)

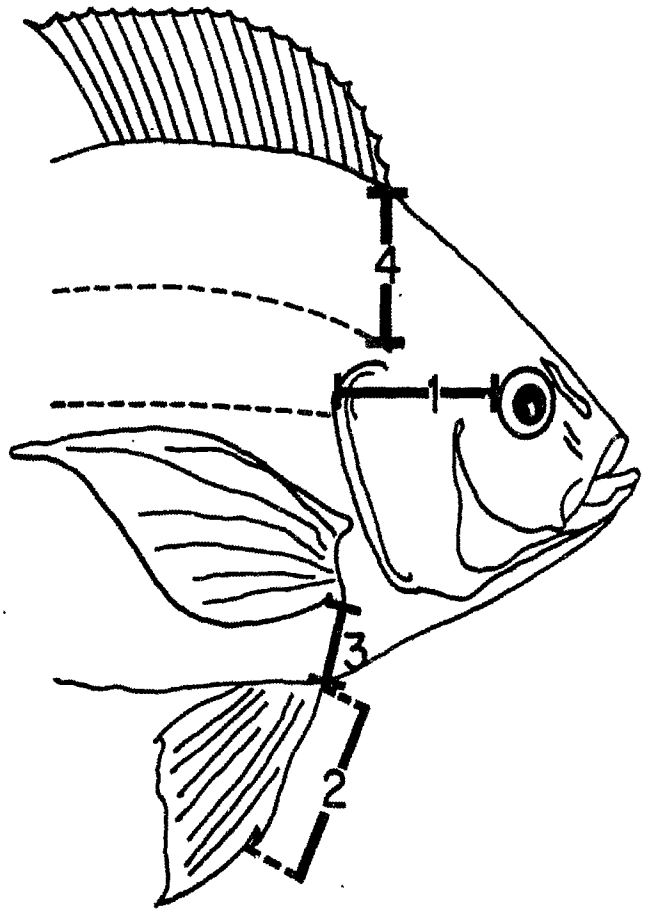


Fig 3.1. Tilapia showing morphological characters (1-4). Characters were measured on right and left (mm) and used in asymmetry analysis: 1) the posterior edge of eye socket to the most posterior edge of the opercular bone 2) the anterior insertion of the pelvic fin to the tip of the first hard ray 3) the ventral insertion of the pectoral fin to the dorsal insertion of the pelvic fin and 4) the lower lateral line to the dorsal insertion of the pectoral fin.

3.2 Descriptive Statistics

Asymmetry 1 values, as estimated by the Palmer and Strobeck ANOVA, are presented in Table 3.2. Seven Side * individual interaction mean squares were not significant relative to measurement error, indicating nonsignificant FA, and were dropped from subsequent analyses. One value was identified as an extreme outlier and also dropped.

TABLE 3.2. Asymmetry 1 values of characters (1-4), calculated as the log (((mean square interaction of sides * individuals minus the error means square)/2) *100). Mean squares are from 2-way ANOVAS of sides and individuals (see text for details).

Character		Regime FE (1)	(2)	(3)	(4)	Average
Line	Family					
A	1	0.505	0.371	0.505	0.538	0.484
	2	0.550	0.643	0.342	0.782	0.607
	3	0.114	-0.155	0.061	0.322	0.118
	4	0.290	0.161	-	0.097	0.190
B	1	0.407	0.398	0.290	0.290	0.350
	2	-0.347	0.061	0.332	0.204	0.126
	3	0.000	-0.046	0.130	0.114	0.056
	4	1.658	-0.046	0.061	0.618	0.315
C	1	0.061	-0.260	0.114	0.061	0.016
	2	-0.222	0.130	1.068	0.190	0.580
	3	-0.097	0.607	0.255	0.498	0.389
	4	-0.046	0.301	0.176	0.190	0.172
D	1	0.041	0.415	0.290	0.371	0.301
	2	0.243	-0.097	0.000	0.190	0.106
	3	-0.301	0.000	0.243	0.301	0.118
	4	0.041	0.371	0.130	0.243	0.214
E	1	0.041	0.161	0.130	0.568	0.279
	2	0.204	0.041	0.842	0.326	0.475
	3	-0.022	-0.222	0.114	-0.097	-0.040
	4	0.204	-0.046	-0.071	0.204	0.093
F	1	0.591	0.447	-0.022	0.407	0.407
	2	0.204	0.290	0.322	0.279	0.276
	3	0.130	0.190	0.176	0.230	0.183
	4	-0.347	-0.071	0.279	0.114	0.051

TABLE 3.2. (continued)

		Regime CE				
A	1	0.447	0.322	0.519	1.145	0.743
	2	0.658	0.190	0.176	0.544	0.443
	3	0.204	0.267	0.362	0.389	0.312
	4	-0.456	0.455	-1.000	0.279	0.114
B	1	0.423	0.580	0.267	0.602	0.488
	2	0.279	0.114	0.097	-0.125	0.114
	3	0.041	0.505	0.000	0.161	0.227
	4	0.217	0.176	0.114	0.628	0.337
C	1	0.204	0.312	0.230	0.031	0.206
	2	0.176	0.332	0.407	0.021	0.258
	3	-0.097	0.021	0.613	0.301	0.298
	4	0.243	0.176	0.190	0.423	0.270
D	1	-0.022	0.633	0.389	0.633	0.477
	2	-0.398	0.312	0.217	0.130	0.134
	3	-0.022	0.217	0.255	0.176	0.169
	4	0.312	0.301	0.407	0.204	0.312
E	1	0.389	-0.022	0.423	-0.097	0.234
	2	0.243	-0.071	0.380	0.322	0.249
	3	0.146	0.407	-0.071	0.423	0.270
	4	0.161	0.470	0.114	0.301	0.284
F	1	0.484	0.470	0.301	0.505	0.447
	2	0.061	0.371	-0.046	0.279	0.197
	3	0.431	0.312	0.279	0.455	0.376
	4	-0.187	0.000	-0.046	0.130	-0.011

3.3 Homogeneity of Variances

Prior to analysing the effect of regime line, strain and cross with parametric ANOVAs, data were checked for homogeneity of variances. Variances were equal between regimes (Table 3.3). Table 3.4 presents the summary statistics of lines within regimes and test for homogeneity of variance among lines. Variance among lines were homogeneous for characters (1), (2) and (4) yet heterogenous for character (3). The latter inequality was due to 1 family in line A. When this outlier was dropped, the variance among lines for character (3) were homogeneous ($F=0.647$, $df=5$, $p=.664$). However, dropping the outlier did not change the results of ANOVA, therefore, it was not deleted from the data set. Part of the problem is that character (3) values do not vary much, therefore the test for homogeneity of variances test is extremely sensitive to outliers. As it is, there appear no real differences in FA values of character (3), the data were also insignificant when analysed using a non-parametric ANOVA, so the violation of variance assumptions was ignored from the outset.

Table 3.5 presents the summary statistics of strains within regimes and test for homogeneity of variance among strains. Within regime FE, variance among strains were

homogeneous for all characters. Within regime CE, variance among strains were homogeneous for all characters except for character (3), this is again due to line A.

Table 3.6 presents the summary statistics of crosses within regimes and test for homogeneity of variance among crosses. Within both regime FE and CE, variance among strains were homogeneous for all characters except for character (3) within regime CE. This was ignored as the outliers made no difference to the ANOVA results of cross and strain.

TABLE 3.3. Regime means of characters (1-4) and average Asymmetry 1 (AVER): (+/-) standard deviation in brackets below means. Test for homogeneity of variances between Regime FE and CE (N=23 or 24).

	Character (1)	(2)	(3)	(4)	AVER
Regime					
FE	0.098	0.156	0.251	0.295	0.245
	(0.266)	(0.259)	(0.262)	(0.199)	(0.178)
CE	0.191	0.284	0.194	0.367	0.311
	(0.239)	(0.193)	(0.318)	(0.253)	(0.160)
[1]Chi Square	0.242	1.822	0.787	1.226	0.261
Prob	0.623	0.177	0.375	0.268	0.610

1 Bartlett's Test for Homogeneity of Variances assuming 1 df

TABLE 3.4. Line mean Asymmetry 1 values of character (1-4) and average Asymmetry 1 (AVER) within regimes: (+/-) standard deviation in brackets below means. Test for homogeneity of variance among lines within regime; (n= 2,3 or 4 families/line/regime).

	Character (1)	(2)	(3)	(4)	AVER
REGIME FE					
Line					
A	0.365	0.255	0.303	0.435	0.350
	(0.202)	(0.337)	(0.225)	(0.293)	(0.234)
B	0.020	0.102	0.203	0.307	0.217
	(0.377)	(0.256)	(0.129)	(0.220)	(0.139)
C	-0.076	0.195	0.403	0.235	0.289
	(0.117)	(0.362)	(0.447)	(0.186)	(0.247)
D	0.006	0.172	0.166	0.276	0.185
	(0.226)	(0.259)	(0.129)	(0.078)	(0.091)
E	0.107	-0.016	0.254	0.259	0.202
	(0.115)	(0.161)	(0.402)	(0.280)	(0.224)
F	0.145	0.214	0.189	0.257	0.229
	(0.385)	(0.217)	(0.153)	(0.121)	(0.150)
[1]Approximate					
F value	1.268	0.416	1.610	1.105	0.673
Prob	0.277	0.837	0.156	0.357	0.644
REGIME CE					
Line					
A	0.463	0.304	0.014	0.589	0.491
	(0.227)	(0.136)	(0.690)	(0.386)	(0.229)
B	0.240	0.344	0.120	0.464	0.306
	(0.158)	(0.233)	(0.110)	(0.262)	(0.140)
C	0.132	0.210	0.360	0.194	0.258
	(0.155)	(0.144)	(0.193)	(0.200)	(0.039)
D	-0.033	0.366	0.317	0.286	0.273
	(0.290)	(0.183)	(0.095)	(0.234)	(0.156)
E	0.235	0.196	0.244	0.349	0.288
	(0.111)	(0.282)	(0.274)	(0.065)	(0.035)
F	0.197	0.288	0.122	0.342	0.252
	(0.318)	(0.203)	(0.194)	(0.171)	(0.205)
[1]Approximate					
F value	0.804	0.357	3.056	1.045	2.403
Prob	0.547	0.877	0.010	0.391	0.036

1 Bartlett's Test for Homogeneity of Variances assuming 5 df

TABLE 3.5. Strain means of characters (1-4) and average Asymmetry 1 (AVER): (+/-) standard deviation in parantheses below strain means, N=no.of families. Test for homogeneity of variance among strains within regime.

	Character									
N	(1)	N	(2)	N	(3)	N	(4)	N	AVER	
				REGIME FE						
Strain										
Nifi	7	0.217	7	0.190	7	0.246	8	0.371	8	0.283
		(0.319)		(0.292)		(0.167)		(0.249)		(0.192)
Israel	8	-0.035	8	0.184	8	0.285	8	0.256	8	0.237
		(0.172)		(0.291)		(0.330)		(0.134)		(0.181)
Hybrid	8	0.126	8	0.099	8	0.221	8	0.258	8	0.215
		(0.264)		(0.216)		(0.284)		(0.200)		(0.177)
[1]Approx F-value		1.128		0.361		1.278		1.204		0.022
Prob		0.325		0.696		0.279		0.302		0.973
				REGIME CE						
Strain										
Nifi	7	0.324	7	0.327	8	0.067	7	0.535	8	0.399
		(0.202)		(0.184)		(0.461)		(0.319)		(0.202)
Israel	8	0.049	8	0.288	8	0.339	8	0.240	8	0.266
		(0.232)		(0.174)		(0.143)		(0.207)		(0.106)
Hybrid	8	0.216	8	0.242	7	0.174	7	0.345	8	0.270
		(0.222)		(0.233)		(0.219)		(0.127)		(0.137)
[1]Approx F-value		0.061		0.321		4.462		2.212		1.395
Prob		0.941		0.725		0.012		0.111		0.249

1 Bartlett's Test for Homogeneity of Variances assuming 2 df

TABLE 3.6. Cross means of characters (1-4) and average Asymmetry 1 (AVER): (+/-) standard deviation in parantheses below cross means, N= # of families, In=inbred, Out=outbred, Hy=Hybrid. Test for homogeneity of variance among crosses within regime.

	Character									
	N	(1)	N	(2)	N	(3)	N	(4)	N	AVER
REGIME FE										
Cross										
In	8	0.144	8	0.225	7	0.360	8	0.335	8	0.320
		(0.281)		(0.325)		(0.346)		(0.251)		(0.225)
Out	7	0.012	7	0.142	8	0.185	8	0.290	8	0.201
		(0.270)		(0.238)		(0.121)		(0.153)		(0.111)
Hy	8	0.126	8	0.099	8	0.221	8	0.258	8	0.215
		(0.264)		(0.216)		(0.284)		(0.200)		(0.177)
[1]Approx										
F-value		0.013		0.620		3.066		0.775		1.536
Prob		0.982		0.537		0.048		0.461		0.216
REGIME CE										
Cross										
In	7	0.262	7	0.251	8	0.187	8	0.392	8	0.375
		(0.236)		(0.138)		(0.504)		(0.354)		(0.196)
Out	8	0.104	8	0.355	8	0.218	7	0.362	8	0.289
		(0.261)		(0.194)		(0.142)		(0.243)		(0.138)
Hy	8	0.216	8	0.242	7	0.174	7	0.345	8	0.270
		(0.222)		(0.233)		(0.219)		(0.127)		(0.137)
[1]Approx										
F-value		0.061		0.321		5.228		2.212		1.395
Prob		0.941		0.725		0.006		0.111		0.249

1 Bartlett's Test for Homogeneity of Variances assuming 2 df

3.4 ANOVA of Regime, Line Strain and Cross

Regime and Line Effect

Data were analysed in a 2 factor ANOVA, regime (FE and CE) and line (A-F), with families nested within lines. Five characters, characters (1-4) and average Asymmetry 1 were analysed in separate ANOVAs (Table 3.7). The regime factor was only significant in the analysis of character (2) (Fig. 3.2) yet almost significant in characters (1) and (4). The effect of line was only significant in character (1) (Fig. 3.3). A post-hoc pairwise comparison showed that the significance of line was due mainly to the difference between Nifi and Israel strains ($F=6.38$, $df= 1,18$, $p<0.05$). The effect of families within lines was significant in characters (1),(3) and (4) and, as a result, in average Asymmetry 1.

Strain Effect

To determine the effect of strain, families were categorized into strain of origin: Nifi, Israel and Hybrids. Characters (1-4) and average Asymmetry 1 were analysed using ANOVAs with 2 factors, regime and strain (Table 3.8). The effect of regime was not significant in any of the analyses. The effect of strain was significant in characters (1) and (4) (Fig. 3.4, 3.5). Pairwise contrasts between strains in both analyses showed that it is Nifi and Israel strain which

cause the significant difference among strains (Character (1): F=9.571, df=1,42, p=0.004; Character (4): F=6.945, df=1,42, p=0.012). The difference between strains in the analysis of character (4) is due to 1 value in line A. Although this value does not cause significant heterogeneity of variance among strains (Table 3.5), when it was dropped from the analysis, the effect of strain was nonsignificant (Strain F-value =2.531,df=2,41,p>0.05). In general, Asymmetry 1 values of characters (1) and (4) of Nifi were higher than that of Israel (Table 3.5).

Cross Effect

To determine the effect of cross, families were categorized into 3 groups: inbreds, outbred and hybrid. The effects of regime and cross were not significant in any of the 5 analyses (Table 3.9).

TABLE 3.7. Regime and Line Effect: Summary of ANOVA results for characters (1-4) and average Asymmetry 1; N=46 for characters (1-4), N=48 for average asymmetry, FwL=family nested within line, MS=mean square, p=probability of significance.

		Character (1) R2=0.773			(2) R2=0.573			(3) R2=0.789		
	DF	MS	F	P	MS	F	P	MS	F	P
Regime	1	0.112	3.565	ns	0.232	4.609	*	0.003	0.085	ns
Line	5	0.716	9.420	**	0.043	0.741	ns	0.113	0.779	ns
FwL	18	0.076	2.406	*	0.058	1.142	ns	0.145	3.837	**
Error	21[1]	0.031			0.050			0.038		

		(4) R^2=0.790			Average Asymmetry 1 R^2=0.774		
	DF	MS	F	P	MS	F	P
Regime	1	0.072	3.113	ns	0.052	3.916	ns
Line	5	0.081	1.080	ns	0.041	0.932	ns
FwL	18	0.075	3.226	**	0.044	3.315	**
Error	21[1]	0.023			0.013		

[1]Degree of freedoms of error term=24 for average Asymmetry 1; ns=not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 3.8. Strain Effect: Summary of ANOVA results for characters (1-4) and average Asymmetry 1; N=46 for characters (1-4) and N=48 for Average Asymmetry 1.

	Character (1) R^2=0.219			(2) R^2=0.101			(3) R^2=0.066		
	DF	MS	F	P	MS	F	P	MS	F	P
Regime	1	0.100	1.854	ns	0.188	3.544	ns	0.034	0.410	ns
Strain	2	0.268	4.946	*	0.032	0.600	ns	0.105	1.255	ns
Error	42[1]	0.054			0.053			0.084		

	(4) R^2=0.172			Average Asymmetry 1 R^2=0.108		
	DF	MS	F	P	MS	F	P
Regime	1	0.067	1.473	ns	0.052	1.896	ns
Strain	2	0.168	3.692	*	0.047	1.711	ns
Error	42[1]	0.046			0.028		

[1]Degree of freedoms of error term=44 for average asymmetry. ns=not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 3.9. Cross Effect: Summary of ANOVA results for characters (1-4) and average Asymmetry 1; N=46 for characters (1-4) and N=48 for average Asymmetry 1.

	Character (1) R^2=0.095			(2) R^2=0.100			(3) R^2=0.023		
DF	MS	F	P	MS	F	P	MS	F	P
Regime 1	0.114	1.819	ns	0.186	3.505	ns	0.041	0.469	ns
Cross 2	0.087	1.393	ns	0.030	0.571	ns	0.025	0.286	ns
Error 42[1]	0.063			0.053			0.088		

	(4) R^2=0.039			Average Asymmetry 1 R^2=0.122		
DF	MS	F	P	MS	F	P
Regime 1	0.058	1.098	ns	0.052	1.926	ns
Cross 2	0.015	0.286	ns	0.057	2.088	ns
Error 42[1]	0.053			0.027		

[1]Degree of freedoms of error term=44 for average asymmetry.
ns not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

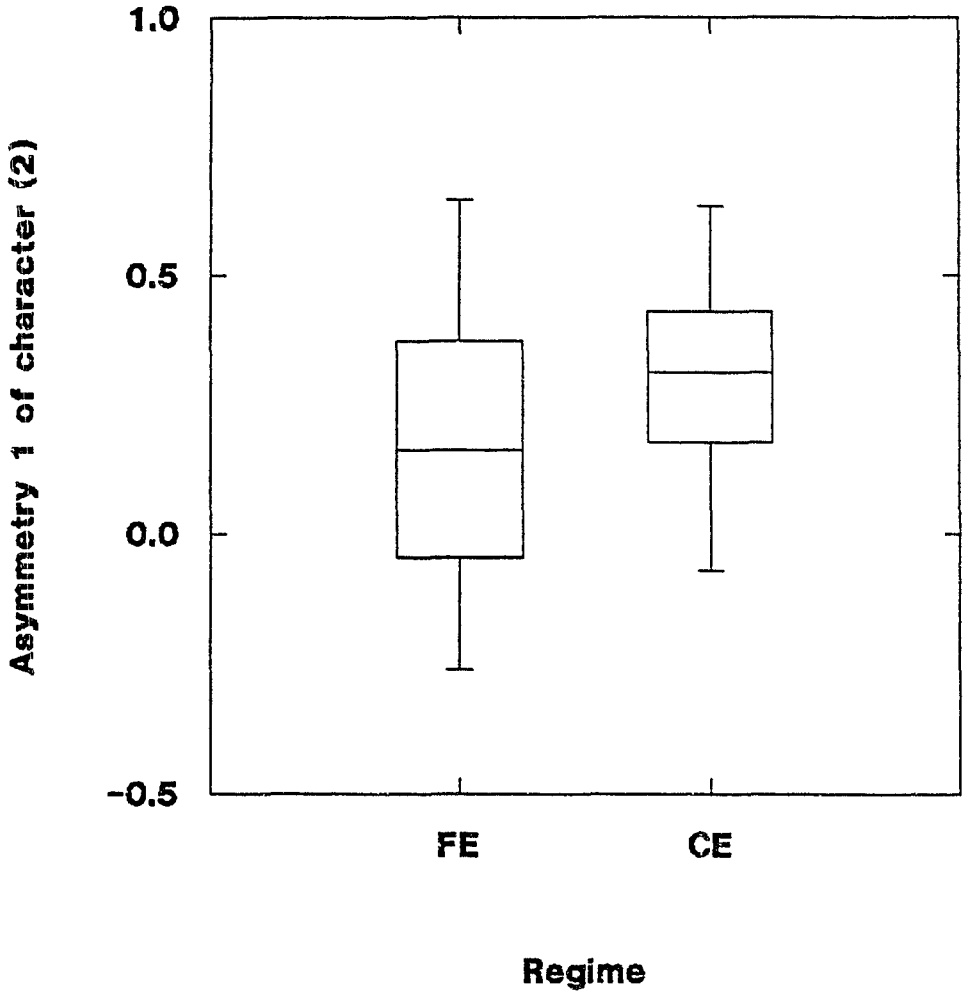


Fig. 3.2. Box plot of Asymmetry 1 of character (2) at each regime. Box represents upper and lower quartiles, horizontal bar in middle is the median, lines stemming from box represent remaining quartiles. N=24 families in each regime.

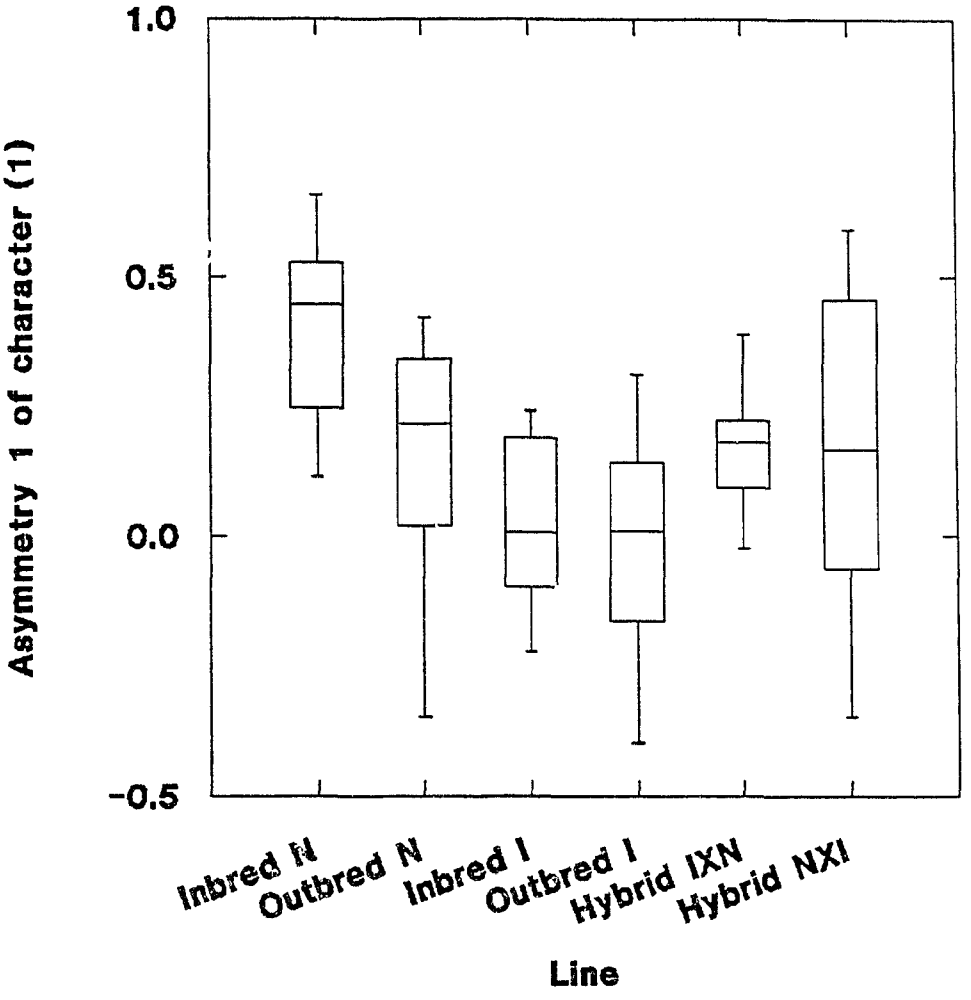


Fig. 3.3. Box plot of Asymmetry 1 of character (1) of Lines. Box represents upper and lower quartiles, horizontal bar in middle is the median, lines stemming from box represent remaining quartiles. *=outliers, N=8 families in each line.

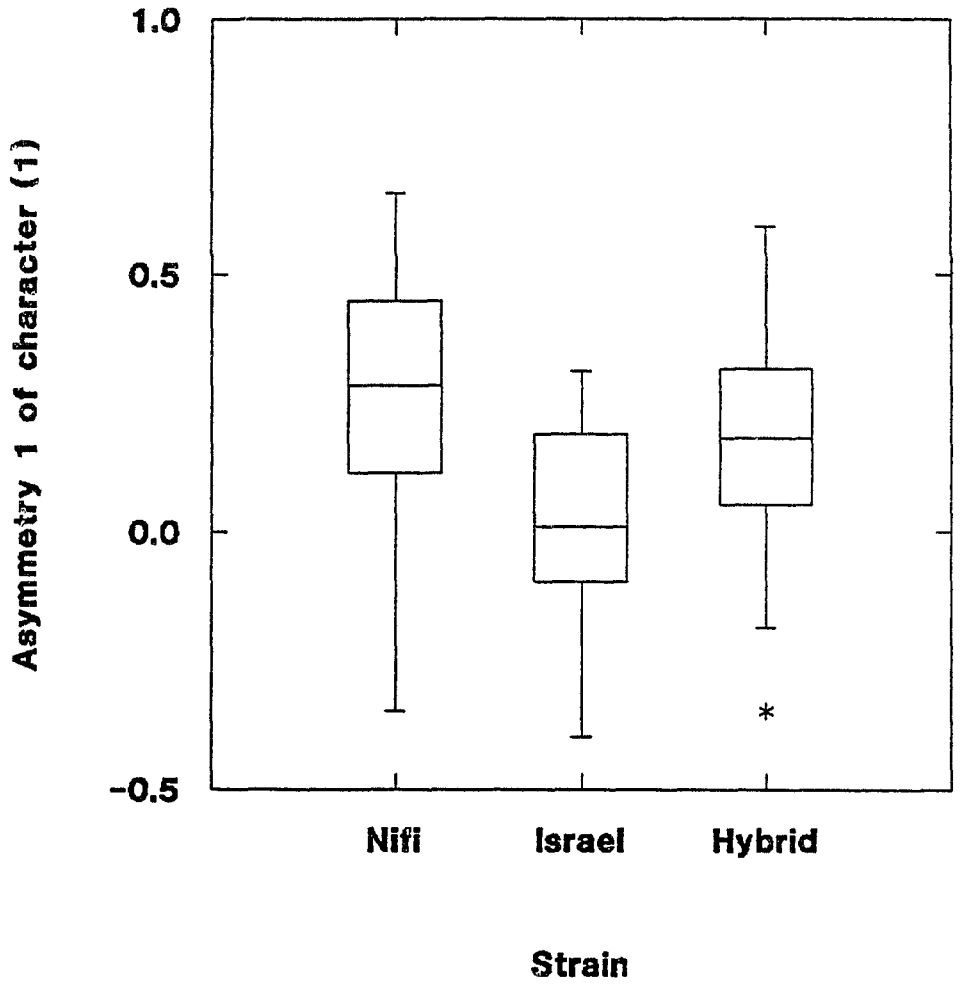


Fig. 3.4. Box plot of Asymmetry 1 of character (1) of Strains. Box represents upper and lower quartiles, horizontal bar in middle is the median, lines stemming from box represent remaining quartiles. *=outliers, N=16 families in each strain.

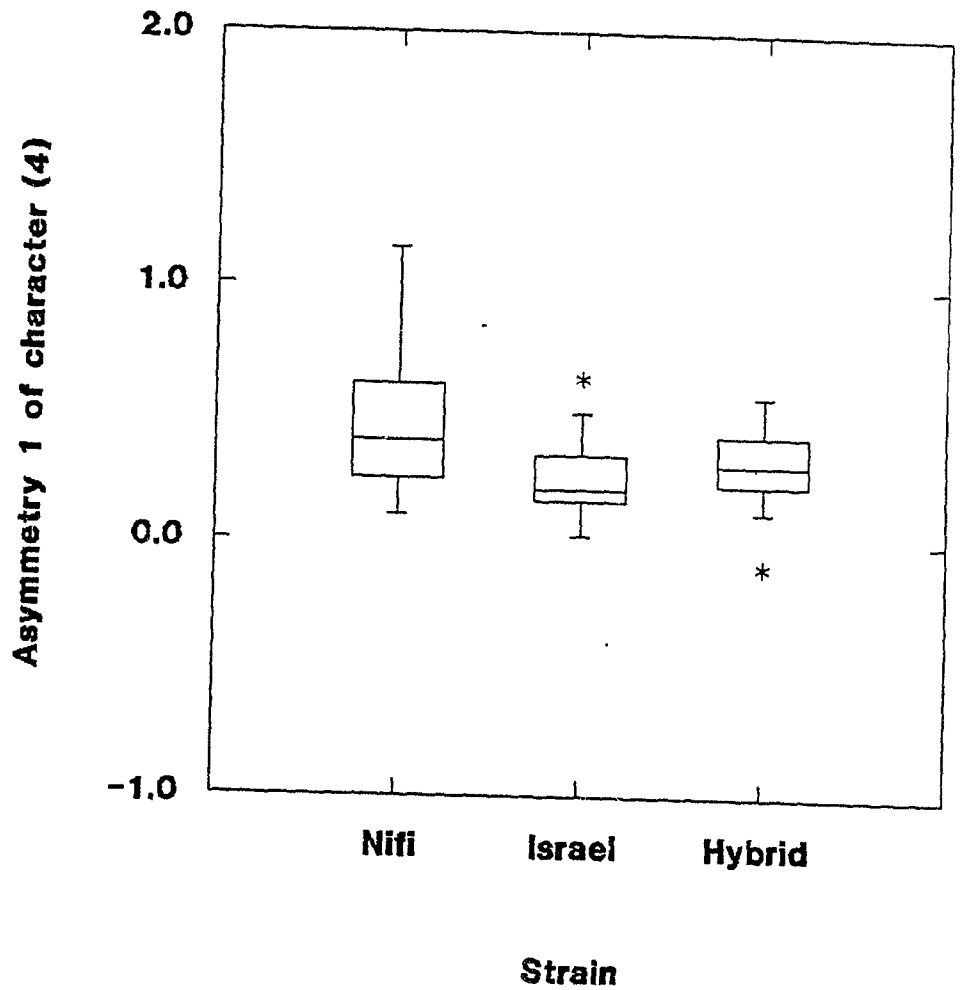


Fig. 3.5. Box plot of Asymmetry 1 of character (4) of Strains. Box represents upper and lower quartiles, horizontal bar in middle is the median, lines stemming from box represent remaining quartiles. *=outliers, N=16 families in each strain.

3.5 Correlation of asymmetry and growth

Correlations among characters (1-4) and growth are presented in Table 3.10. Growth was measured as specific growth as in Chapter 2 (difference between log final and log initial standard length, divided by number of days). Kendall's coefficient of concordance among characters (1-4) (which represents association among characters) was 0.104 (p=n.s.), indicating that integration among characters was low. Overall, pooling regimes, (N=48) correlations among characters (1-4) ranged from -0.056 to 0.543. Within regime FE, correlation between characters ranged from -0.144 to 0.727. Within regime CE, family correlations ranged from -0.126 to 0.497. In effect, the lack of significant correlations may be due to the small range of asymmetry values. Each character is a fairly independent estimate of FA, therefore an average Asymmetry is an indication of the composite FA of these particular characters. If it is desired that an average Asymmetry represent an actual level of FA, then average asymmetry would have to be constructed based on the variance and the correlation among characters.

No character was significantly correlated with growth when regimes are pooled. Within regime CE, both character (1) and average Asymmetry 1 show weak relationships with growth; scatterplots show that one point influences these relationships (Fig. 3.6, 3.7). In the case of character (1),

the correlation was strengthened from -0.377 to -0.615 ($p<0.001$), when the outlier was excluded. In the case of average Asymmetry 1, the correlation was weakened from -0.400 to -0.277, when the outlier was excluded.

TABLE 3.10. Pearson product-moment correlation matrix of pooled regimes (N=40 among characters (1-4), N=46 between each character and growth, except for Average (AVER) where N=48) and within regime FE (N=21 among characters (1-4); N=23 or 24 for each character and growth) and CE (N=19 for characters (1-4); N=22or 23 for each character and growth).

	Character (1)	(2)	(3)	(4)	AVER
		POOLED REGIMES			
Character					
(2)	0.328*				
(3)	-0.056	0.061			
(4)	0.503**	0.543***	0.106		
Growth	-0.017	0.080	-0.079	0.051	-0.105
		REGIME FE			
(2)	0.423				
(3)	-0.144	0.163			
(4)	0.497*	0.727***	0.162		
Growth	0.030	-0.023	-0.296	0.141	-0.142
		REGIME CE			
(2)	0.142				
(3)	0.105	-0.126			
(4)	0.497*	0.262	0.037		
Growth	-0.377[1]	-0.207	0.177	-0.203	-0.400
[1]Excluding Outlier	-0.615**				

* Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

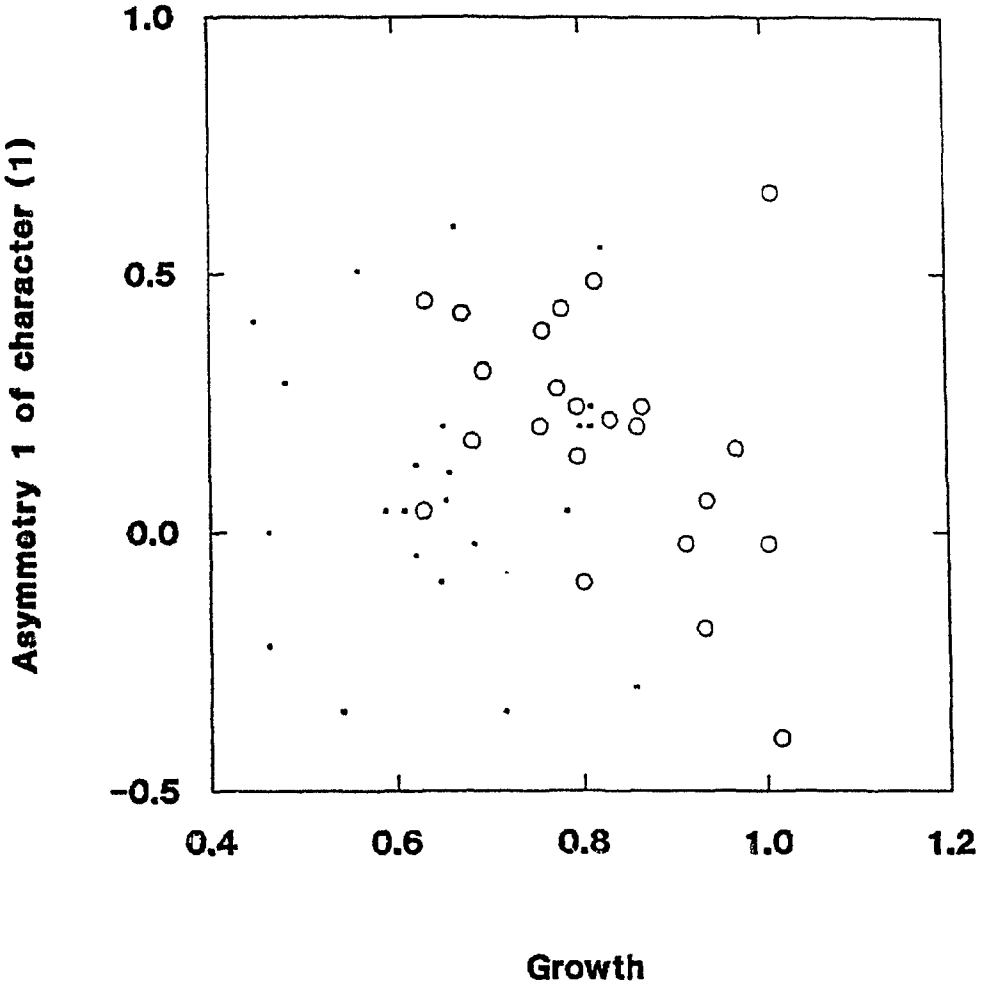
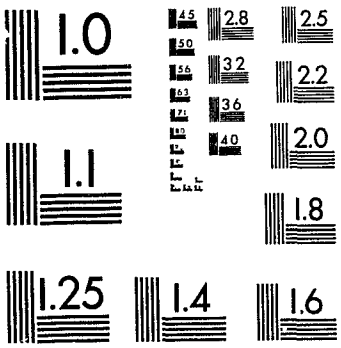


Fig. 3.6. Asymmetry 1 of character (1) plotted against Growth (% log mm/day). Filled circles= regime FE, open circles= regime CE.

2



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No 2)

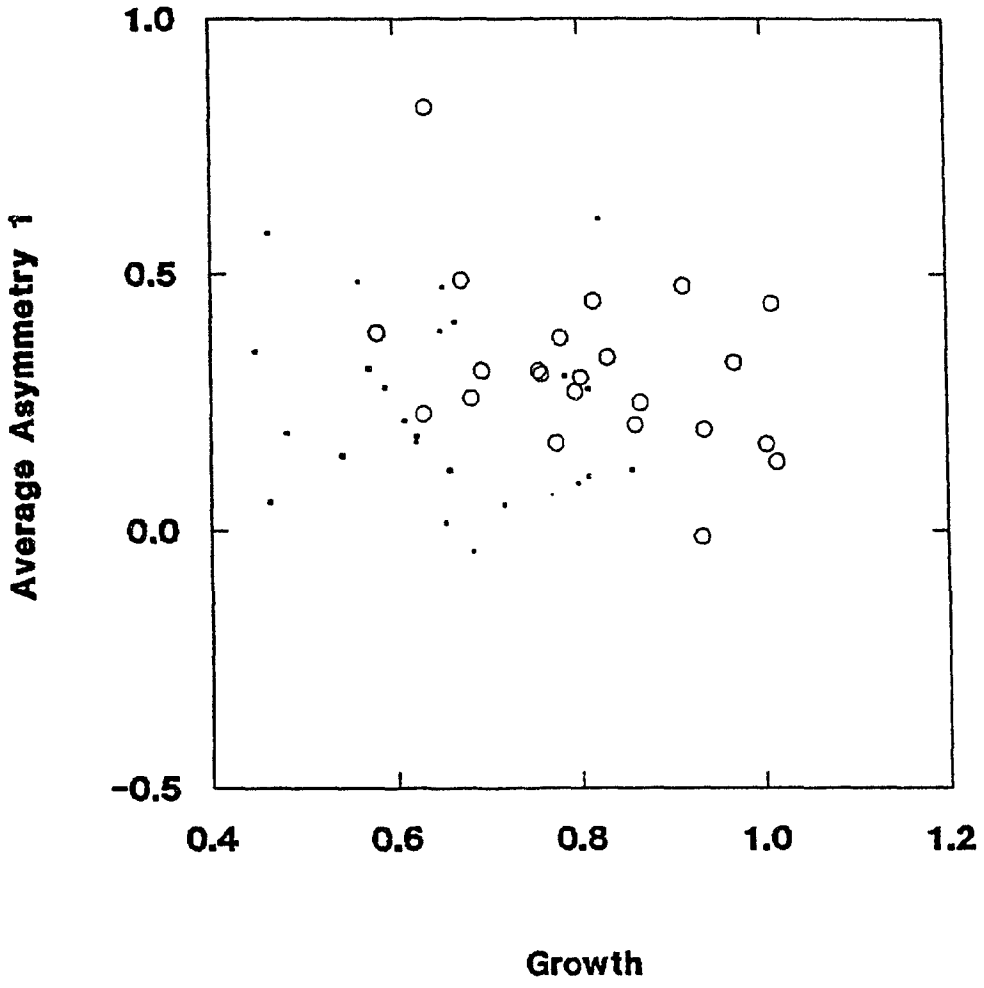


Fig. 3.7. Average Asymmetry 1 plotted against Growth (% log mm/day). Filled circles= regime FE, open circles= regime CE.

3.6 SUMMARY of Method 1

Developmental stability, as measured by asymmetry of 4 bilateral characters was compared between regimes, among lines, among strains and among crosses. The effect of regime was significant in the analysis of character (2) and almost so for characters (1) and (4). The effect of line (after accounting for variation among families within lines) was significant in characters (1). The significant difference among lines is derived from difference between Nifi and Israel as shown by pairwise contrasts and confirmed by analysis of strain effects. Families within lines was significant in characters (1),(3),(4) and average Asymmetry 1. There was a significant effect of strain for character (1). There was no effect of cross, that is, inbreds, outbreds and hybrids do not have significantly different levels of asymmetry in any of the characters.

The correlation between characters (1) and (4) was significantly positive. The correlation between character (2) and character (4) was significantly positive except within regime CE. Character (1) was significantly negatively correlated with growth.

4 RESULTS: Method 2

4.1 Descriptive Statistics

As described earlier in the text, bilateral asymmetry of an individual has 3 sources: directional asymmetry, antisymmetry and FA. The 3 forms of asymmetry differ in their expected distribution properties. 1) The normal distribution of a population showing directional asymmetry has a mean above or below 0. This occurs when one side of an individual is consistently bigger, for example, the right arm is typically stronger and larger in human populations. 2) The distribution of a population showing antisymmetry is bimodal or platykurtotic and has a mean of 0. This occurs when either the left or the right side of an individual is larger in roughly equal frequencies, for example 50% right-handed and 50% left-handed individuals in one population. 3) A population showing FA has a normal distribution and a mean of 0. The variance is 0 if all individuals are perfectly symmetric (e.g. completely ambidextrous using the right/left handed example). A variance larger than 0 measures the deviation from perfect symmetry caused by variable expression of genes during development. FA differs from antisymmetry in that either the left or the right side of an individual can be larger but the frequency is random (Palmer and Strobeck 1986). Before

analysing signed differences for the presence of FA, the data were checked for presence of directional asymmetry, antisymmetry, and dependency of asymmetry variance on size.

Directional asymmetry

The expected mean of signed differences in a population of bilateral organisms is 0, meaning that, on average, left and right sides are of equal size. Deviation from 0, in one direction, indicates directional asymmetry. The means of characters (1-4) were not significantly different from 0, as indicated by the fact that 0 falls within the confidence limits (Table 3.11).

Antisymmetry

Distributions of signed differences were tested for normality using the Kolmogorov-Smirnov 1-sample test which tests a distribution of sample values against a theoretical distribution (Siegel and Castellan 1988). Frequency distribution plots of 1920 signed differences of characters (1-4) were significantly different from a standard normal distribution. Table 3.12 shows that distributions of signed differences of characters (1-4) are not normal, yet frequency histograms of each character do not appear obviously platykurtotic or bimodal (Fig. 3.8-3.11). The non-normality seems to be caused by a significant number of outliers in both tails of the distribution, i.e., leptokurtosis. In any event, the frequency distributions were neither platykurtotic nor bimodal so the effect of

antisymmetry was not considered important. Ross and Robertson (1990) studied the developmental stability of two fire ants (Solenopsis invicta and S. richteri) and also observed significant non-normality of character frequency distributions of differences yet no platykurtosis nor bimodality.

Size dependency of variance of signed differences

If the variance increases with size, then the data would have to be size-corrected in order to equalize variances. Left values were plotted against right values to examine the scatter around the axis of symmetry as a measure of the variance. Figures 3.12-3.15 show that the variance around the axis of symmetry does not increase in any of the characters. The correlation of signed differences and character size were low but did reach significance in charaters (1) and (3) ($r=0.103$, $r=-0.233$ respectively $p<0.001$,$df=1918$), perhaps due to the large number of degrees of freedom. The effect of character size on the variance of signed differences is not strong, therefore the characters were not size-corrected.

In conclusion, signed differences are not overly influenced by directional asymmetry, antisymmetry nor size differences among individuals.

TABLE 3.11. Mean, (+/-) standard deviations and confidence limits for signed differences of characters (1-4) (N=1920 for each character).

Character	(1)	(2)	(3)	(4)
Mean	-0.134	-0.042	-0.145	-0.066
Standard deviation	0.207	0.224	0.229	0.264
Confidence limits	0.406	0.439	0.449	0.517

TABLE 3.12. Kolmogorov-Smirnov one sample test using standard normal distribution for characters (1-4): N=# of individuals Maxdif=maximum difference between normal and observed distributions.

Character	N	Maxdif	Probability (2-tail)
(1)	1920	.374	.000
(2)	1920	.342	.000
(3)	1920	.375	.000
(4)	1920	.324	.000

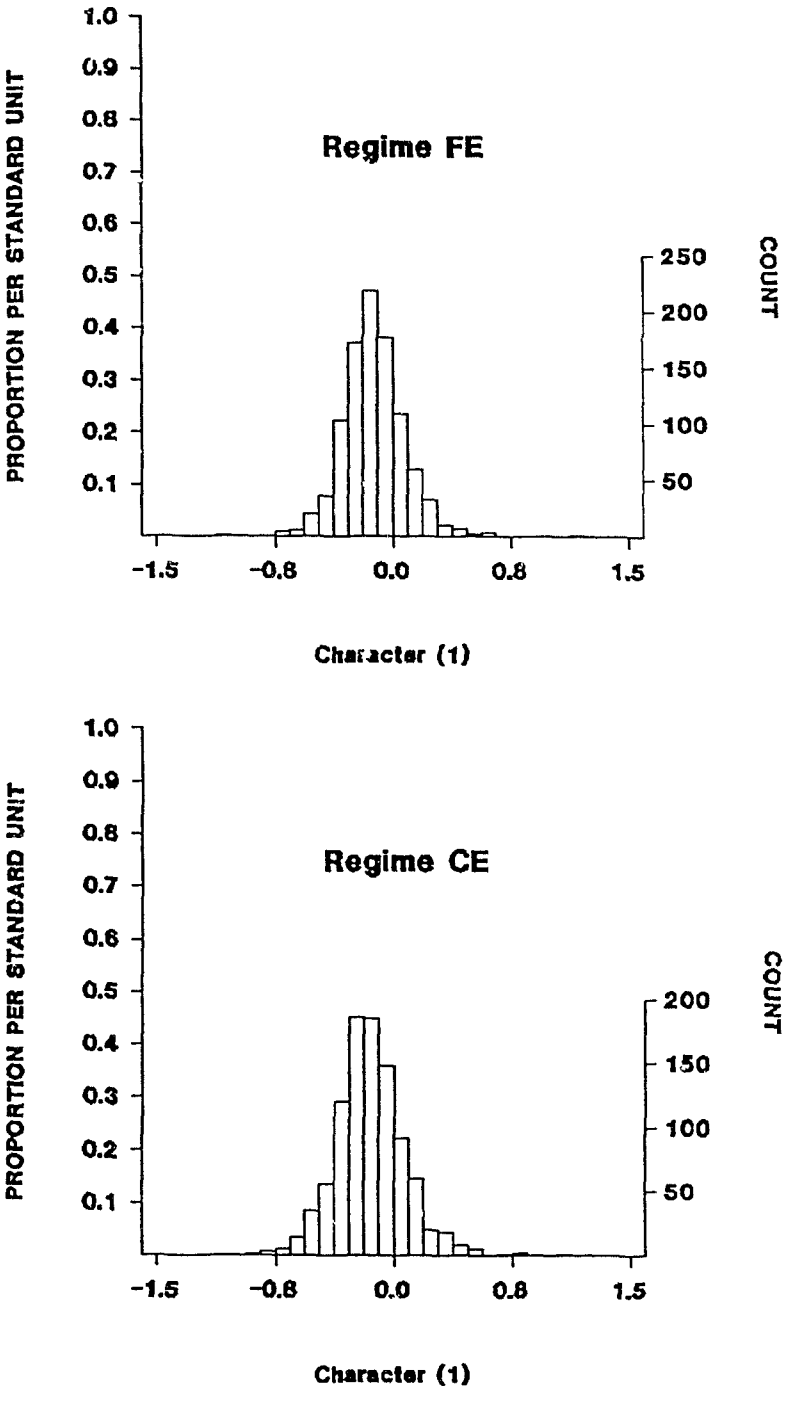


Fig. 3.8. Frequency distribution of signed differences (mm) between right and left of character (1) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

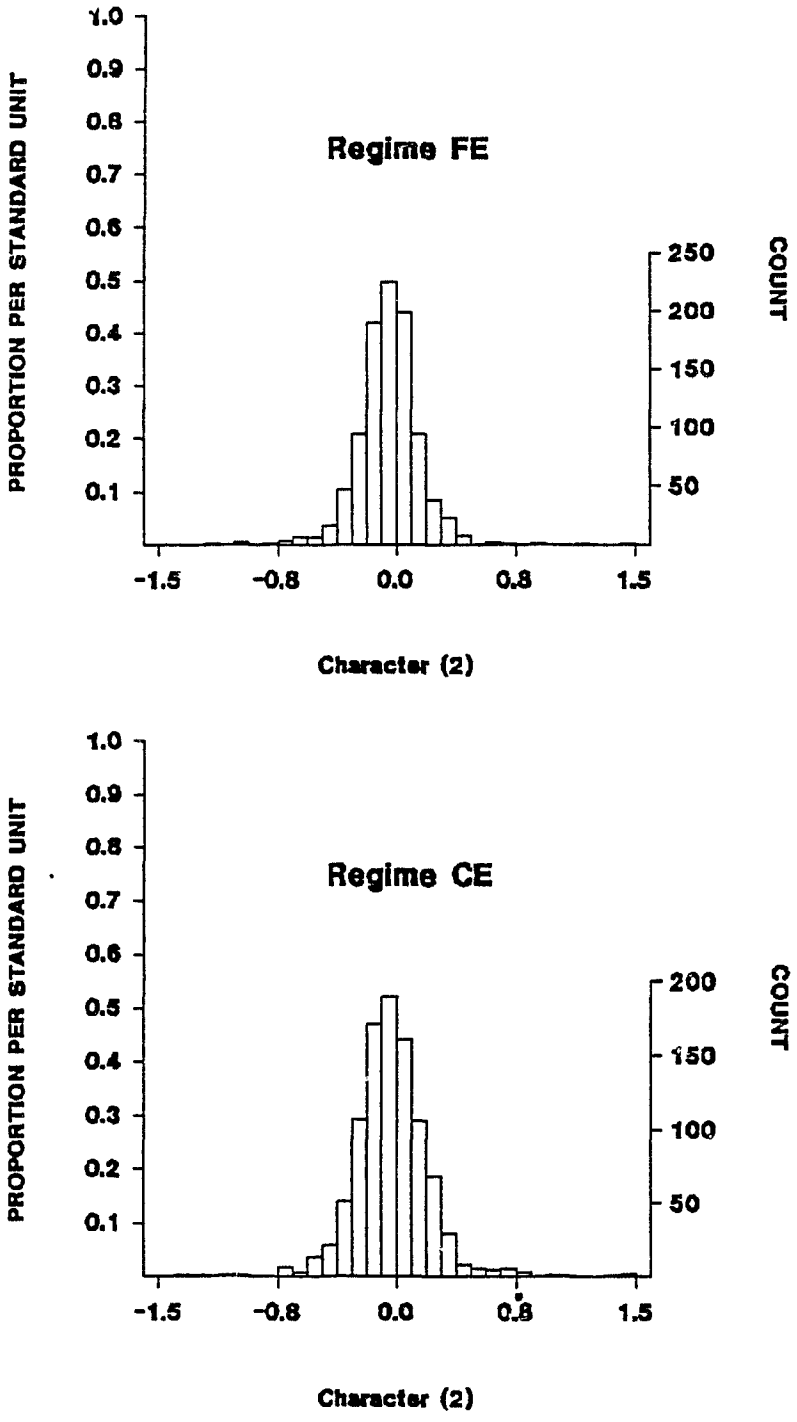


Fig. 3.9. Frequency distribution of signed differences (mm) between right and left of character (2) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

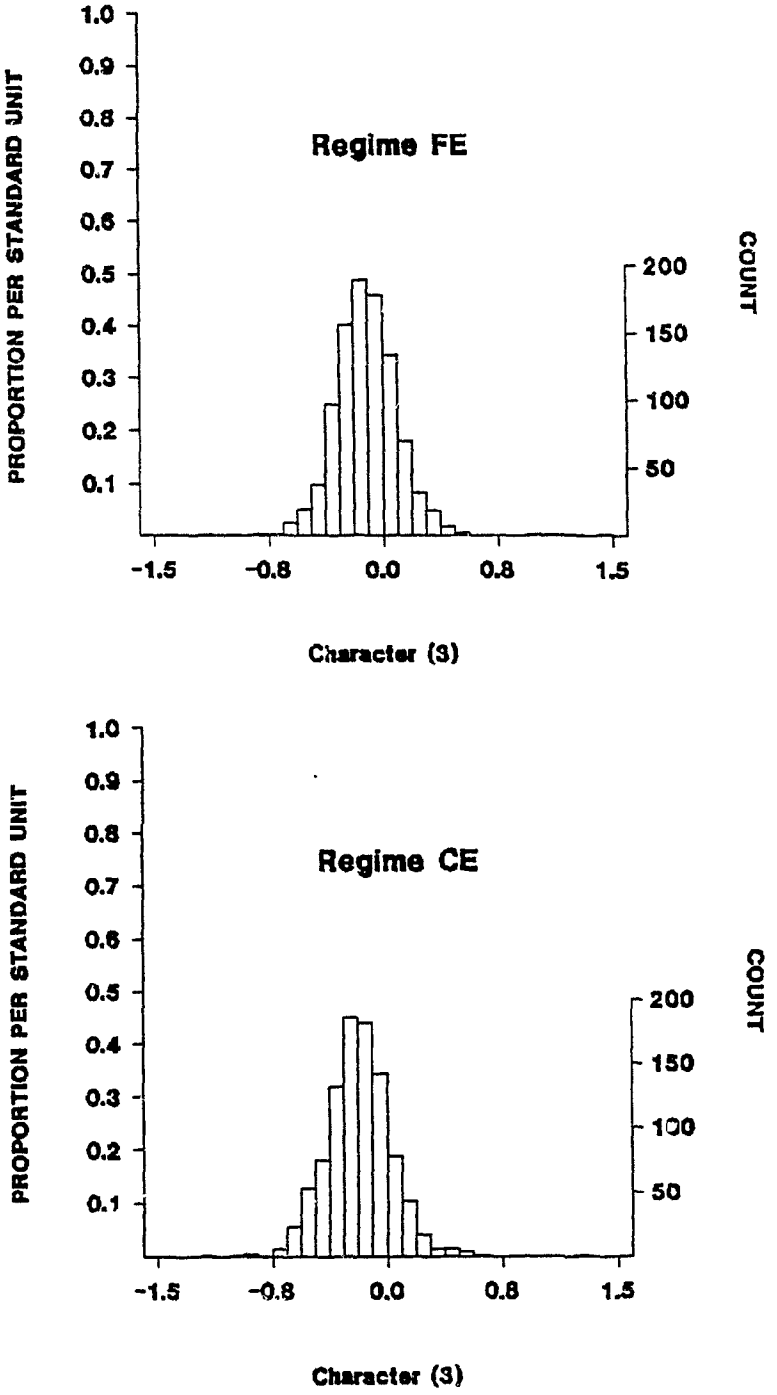
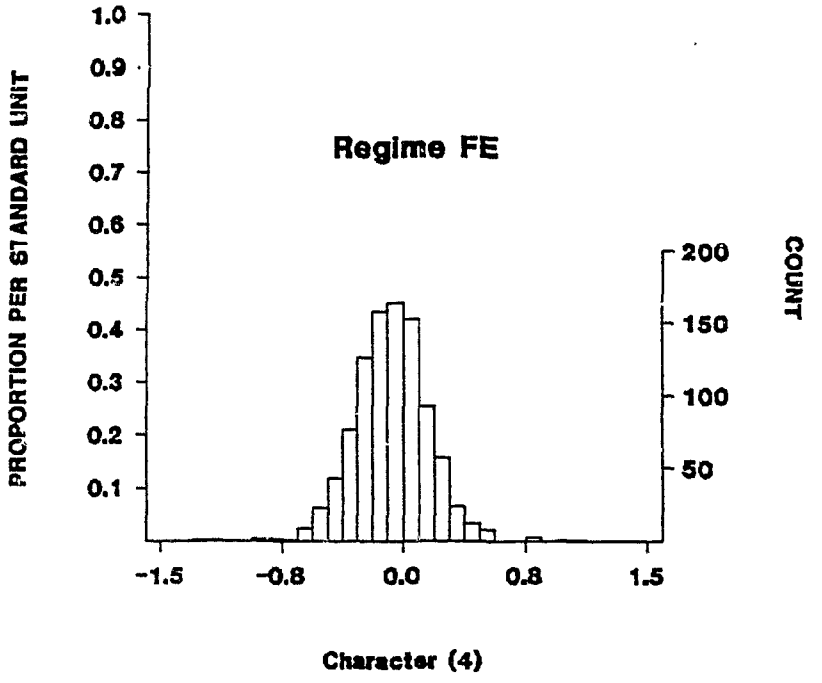


Fig. 3.10. Frequency distribution of signed differences (mm) between right and left of character (3) in each regime.Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.



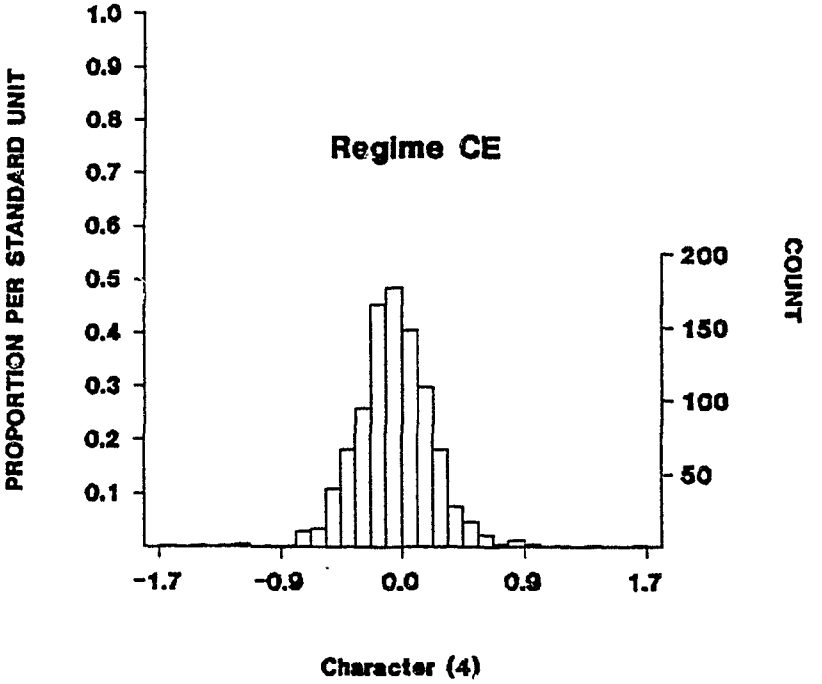


Fig. 3.11. Frequency distribution of signed differences (mm) between right and left of character (4) in each regime.Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

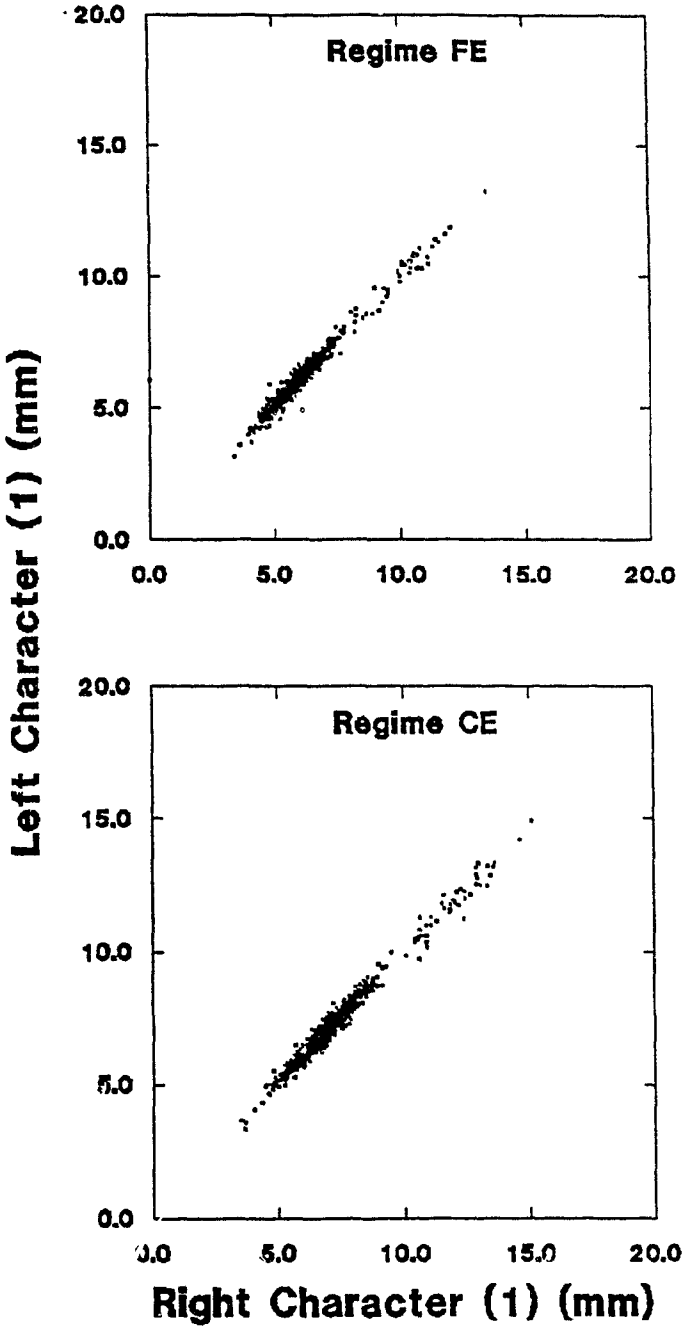


Fig. 3.12. Left character (1) plotted against right character (1) (mm) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

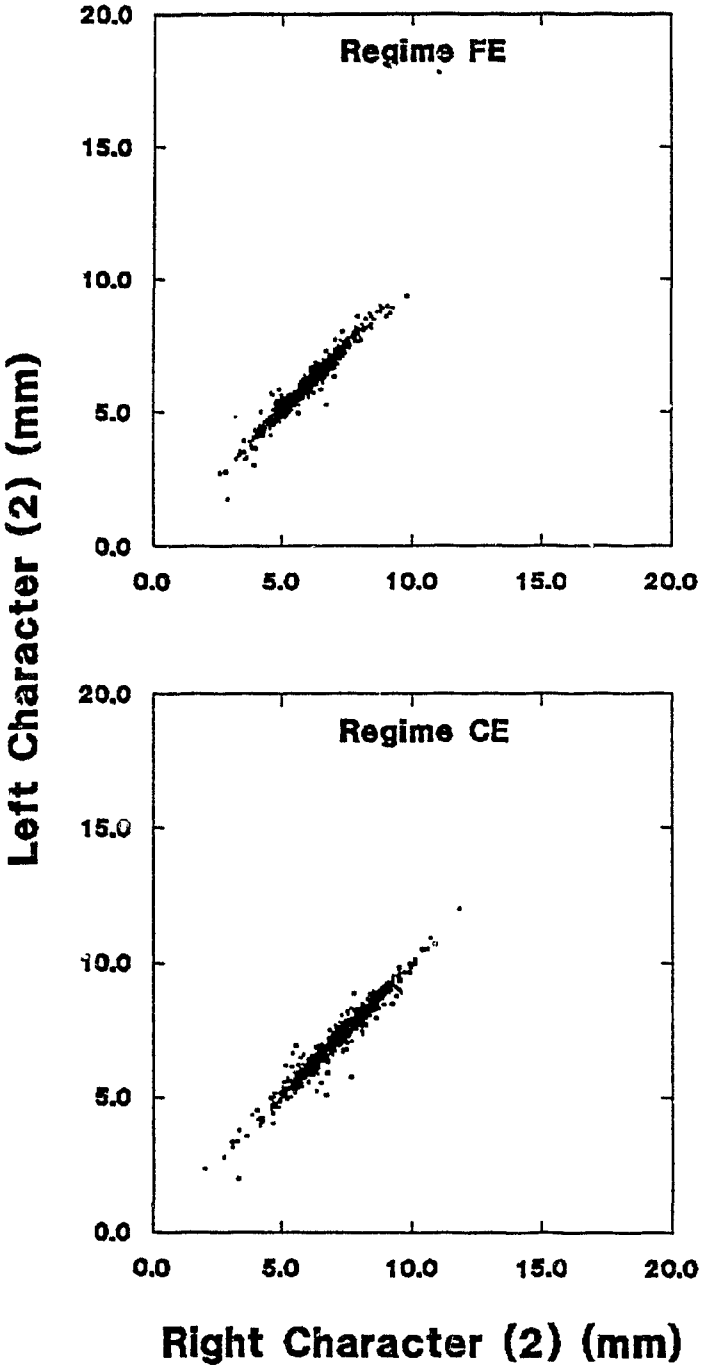


Fig. 3.13. Left character (2) plotted against right character (2) (mm) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

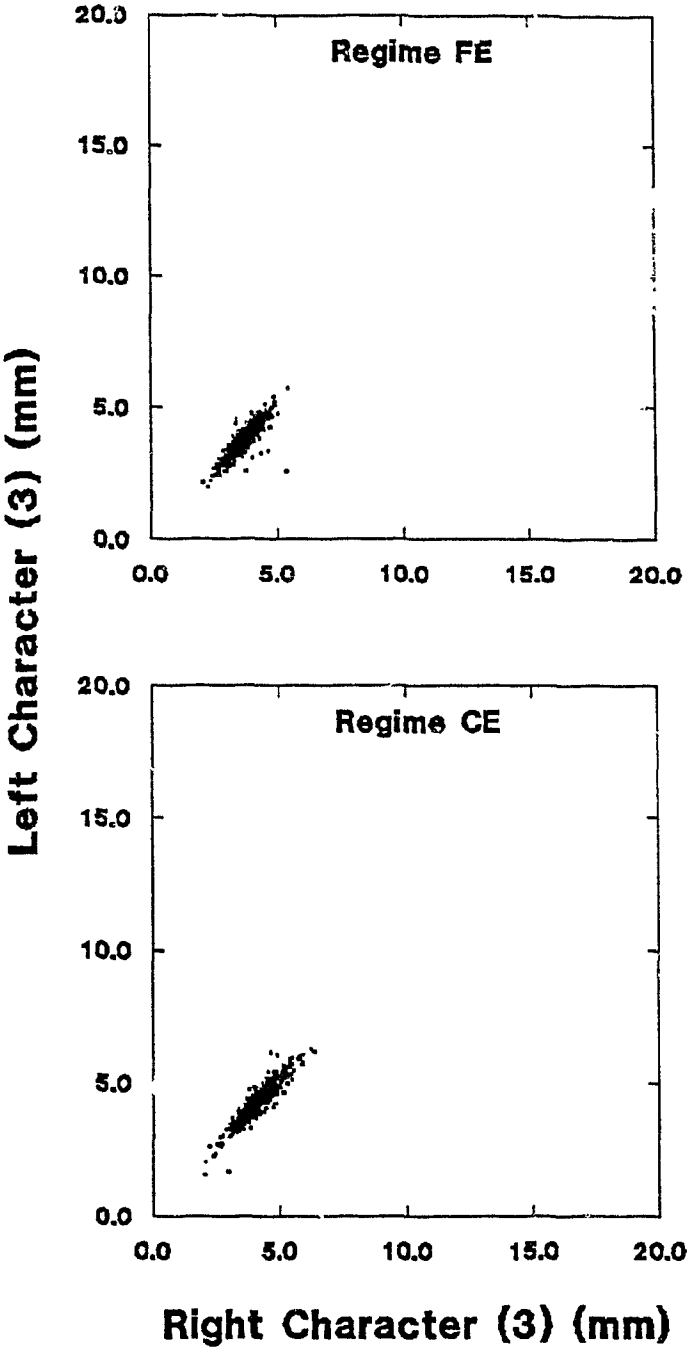


Fig. 3.14. Left character (3) plotted against right character (3) (mm) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

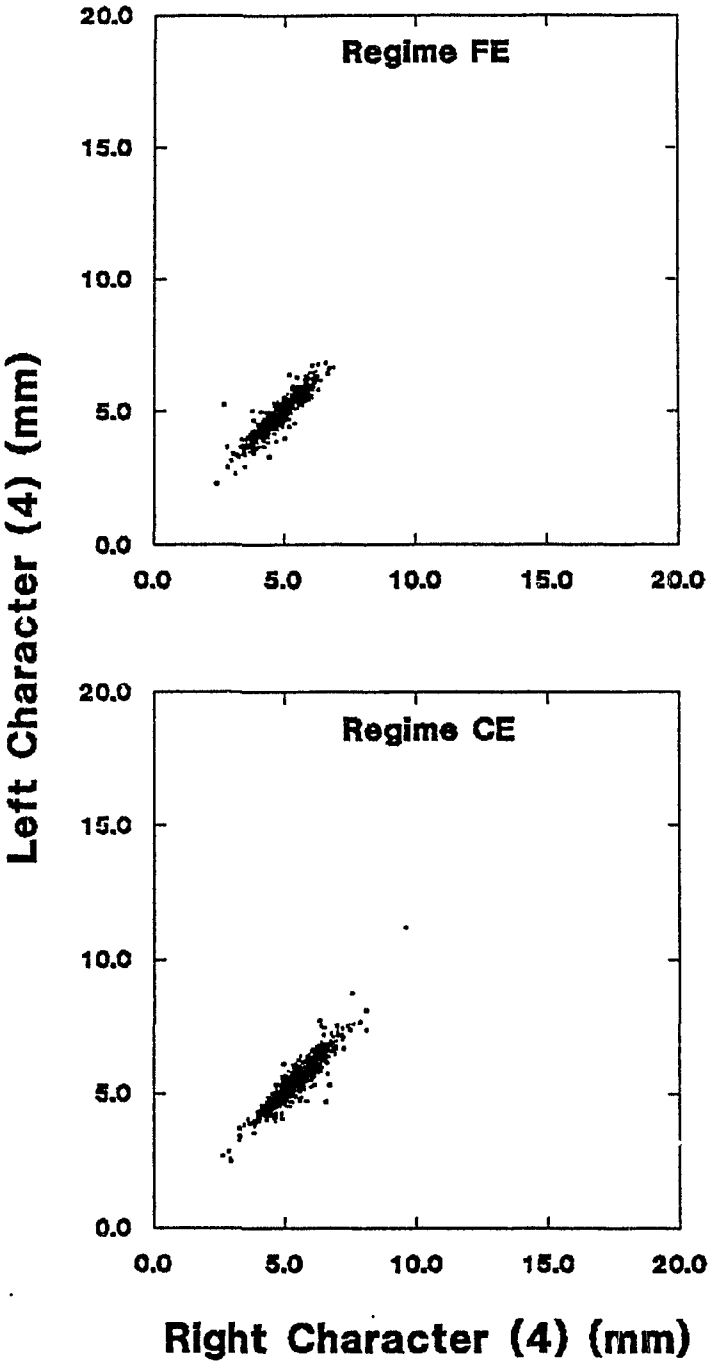


Fig. 3.15. Left character (4) plotted against right character (4) (mm) in each regime. Regime FE=fluctuating low-high-low protein regime. Regime CE=constant high protein regime.

4.2 Homogeneity of Variance of Asymmetry 2

Given that the frequency distribution of individual signed differences has an expected mean of 0, the variance reflects the level of population asymmetry. A relatively larger within-group variance indicates that a given group contains more highly asymmetric individuals (and thus lower developmental stability) than another group. A higher level of the variance of differences at one factor level indicates a higher level of population asymmetry relative to another factor level. The variances of individual asymmetries of characters were compared between regimes, among lines, among strains and among crosses, using Bartlett's Test for Homogeneity of Variance. The effect of lines, strains and crosses were analysed within each regime as in some instance, the ranking of lines, strains and crosses were different in each regime.

Between Regimes

The variances of all characters were significantly different between regimes (Table 3.13). The variance in regime CE was larger than regime FE, except in character (3). For characters (1) (2) and (4), fish fed a constantly higher protein diet showed a decreased developmental stability. This is contrary to what I expected as fish in a presumably more stressful environment are expected to show

decreased developmental stability. The result suggests that the data were influenced by character size. However, I judged that the effect of size was not strong enough to warrant transforming the data. Correcting for size, by dividing the signed differences by character size, can lead to misleading results as the larger individuals will have proportionally smaller variances than smaller individuals (R. Palmer, Univ. of Calgary, pers. commun.). Note that the largest difference between regime Asymmetry 2 values is in character (2), as it is in Asymmetry 1. The signed differences of character (2) are not significantly correlated with character size, nor does the variance around the axis of symmetry increase in scatterplots of left vs right characters (Fig. 3.13). Therefore it appears that asymmetry of character (2) is larger in regime CE than in regime FE.

Among Lines

Homogeneity of variance of characters (1-4) were compared among lines (Table 3.14, Fig. 3.16). Within each regime, individuals were pooled within lines. In regime FE, the variances among lines were heterogeneous for all characters. The order of lines from largest to smallest variances differs among characters (1-4). In order to gain an overview of the relative line order, variances of lines were ranked for each character and then summed over

character. In order of descending variance: A>C>B>F>E>D. Inbred lines were more asymmetric than other lines, indicating a lower developmental stability of inbred fish. In regime CE, the variances among lines were heterogeneous for all characters. The order of lines from largest to smallest variances, differs among characters (1-4). Overall, the order of lines is as follows: A>B=D=F>E>C. The results of line C were conflicting. Line C in regime FE had relatively higher levels of asymmetry, yet relatively low in regime CE.

In summary, in regime FE, inbred lines A and C have higher levels of asymmetry, reflecting a lower developmental stability. In regime CE, inbred line A was the most asymmetric whereas C was the least. Inbred line C in regime FE had low developmental stability which either does not show up or is non-existent in regime CE.

Among Strains

Within both regimes, the variances among strains were heterogeneous for all characters (Table 3.15, Fig. 3.17). Within regime FE, the variance of Nifi was largest in characters (1) and (2). The variance of Israel was largest in characters (3) and (4). The variance of hybrids was smallest in all characters. Within regime CE, the variance of Nifi was largest in all characters, whereas the variance

of Israel is smallest in characters (1), (2) and (3). In general, the Nifi strain had more highly asymmetric individuals compared to the other 2 strains.

Among Crosses

Within both regimes, the variances among crosses were heterogeneous for all characters, except character (2) within regime CE (Table 3.16, Fig. 3.18). In all instances, the variance of inbreds was largest, indicating that inbred individuals were, on average, more asymmetric than outbreds or hybrids.

TABLE 3.13. Variances (Asymmetry 2) of regimes. Test for homogeneity of variance between Regime FE (N=960) and CE (N=960).

Character	(1)	(2)	(3)	(4)
Regime				
FE	0.038	0.039	0.054	0.061
CE	0.048	0.061	0.048	0.078
[1]Chi Square	13.262	45.784	3.324	14.132
Prob	0.000	0.000	0.000	0.000

1 Bartlett's Test for Homogeneity of Variances assuming 1 df

TABLE 3.14. Variances of lines within regime and test for homogeneity of variance among lines A-F (N=160 in each line/regime).

	Character (1)	(2)	(3)	(4)
		REGIME FE		
Line				
A	0.075	0.048	0.063	0.087
B	0.031	0.043	0.038	0.048
C	0.023	0.045	0.086	0.085
D	0.026	0.036	0.035	0.049
E	0.031	0.025	0.060	0.047
F	0.040	0.039	0.032	0.042
[1]Chi Square	81.299	20.154	62.545	43.225
Prob	0.000	0.000	0.000	0.000
		REGIME CR		
Line				
A	0.086	0.093	0.075	0.161
B	0.048	0.051	0.031	0.064
C	0.034	0.036	0.041	0.050
D	0.025	0.066	0.045	0.054
E	0.045	0.062	0.042	0.064
F	0.044	0.056	0.044	0.058
[1]Chi Square	68.781	37.769	35.005	88.183
Prob	0.000	0.000	0.000	0.000

1 Bartlett's Test for Homogeneity of Variances assuming 5 df

TABLE 3.15. Variances of strains within regime: Test for homogeneity of variance among strains (N=320 in each strain/regime).

	Character (1)	(2)	(3)	(4)
		REGIME FE		
Strain				
Nifi	0.054	0.046	0.053	0.067
Israel	0.024	0.041	0.061	0.071
Hybrid	0.035	0.032	0.046	0.045
[1]Chi				
Square	49.777	10.354	6.405	19.402
Prob	0.000	0.006	0.041	0.000
		REGIME CE		
Strain				
Nifi	0.068	0.072	0.055	0.112
Israel	0.029	0.051	0.043	0.057
Hybrid	0.046	0.059	0.043	0.064
[1]Chi				
Square	53.451	9.345	5.993	43.279
Prob	0.000	0.009	0.050	0.000

1 Bartlett's Test for Homogeneity of Variances assuming 2 df

TABLE 3.16. Variances of crosses within regime and test for homogeneity of variance among crosses (N=320 in each strain/regime).

	Character (1)	(2)	(3)	(4)
		REGIME FE		
Cross				
Inbred	0.049	0.046	0.075	0.086
Outbred	0.028	0.040	0.036	0.049
Hybrid	0.035	0.032	0.046	0.045
[1]Chi				
Square	24.713	11.143	43.759	41.741
Prob	0.000	0.004	0.000	0.000
		REGIME CE		
Cross				
Inbred	0.061	0.065	0.061	0.108
Outbred	0.036	0.059	0.038	0.060
Hybrid	0.046	0.059	0.043	0.064
[1]Chi				
Square	20.869	0.964	20.041	33.883
Prob	0.000	0.618	0.000	0.000

1 Bartlett's Test for Homogeneity of Variances assuming 2 df

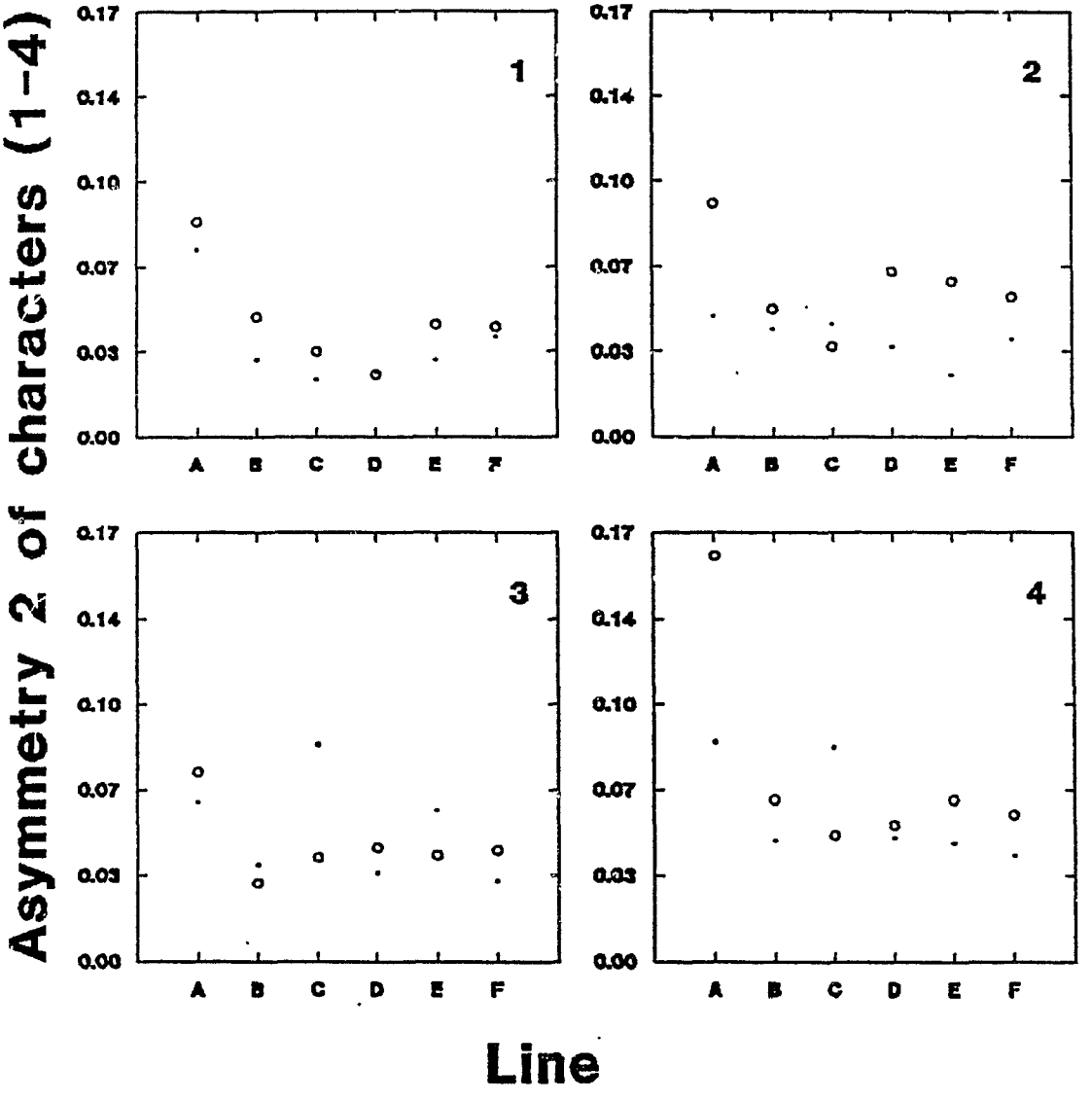


Fig. 3.16. Asymmetry 2 of characters (1-4) of lines. Filled circles= regime FE, open circles= regime CE. N=4 families in each line/regime.

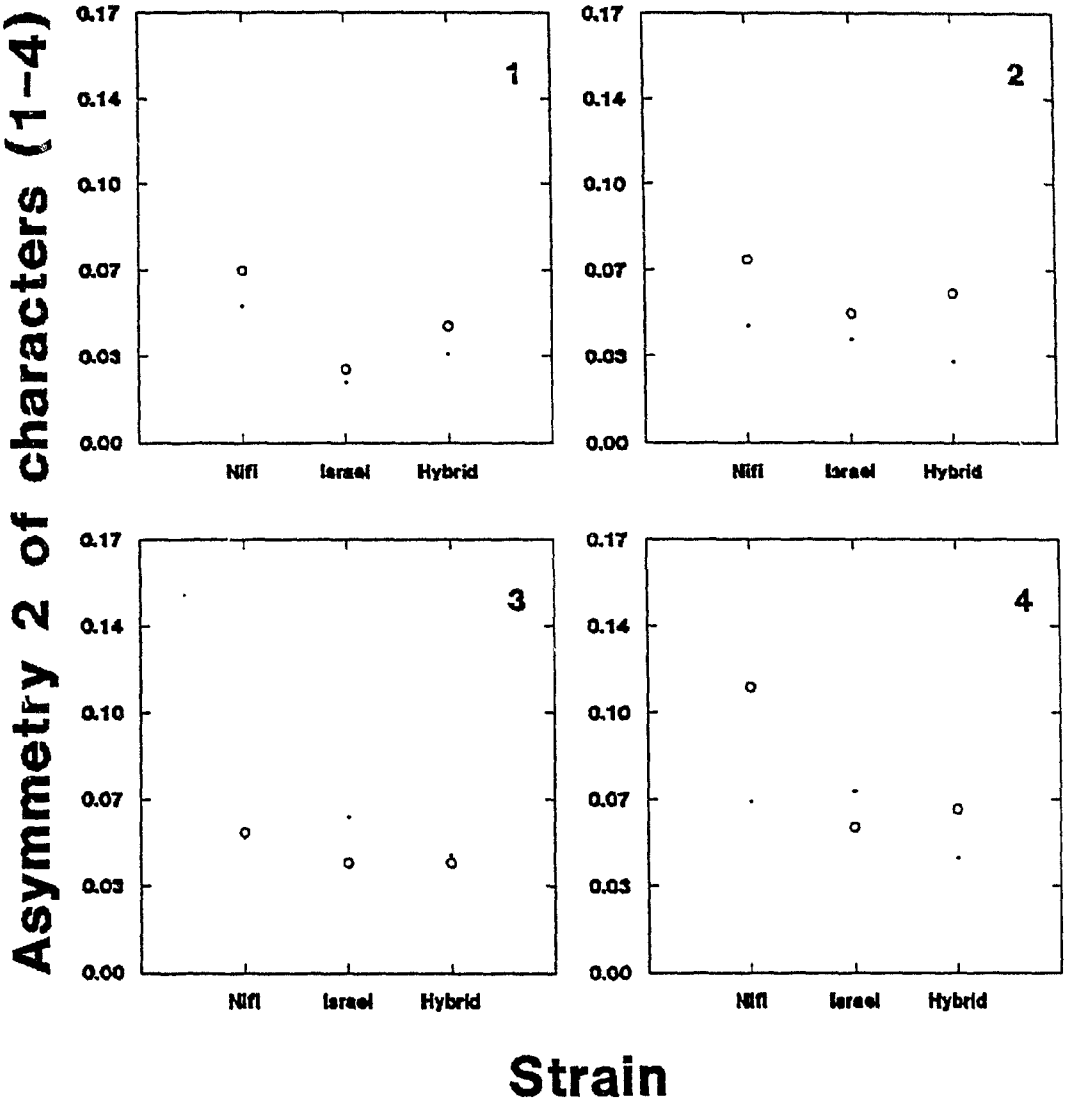


Fig. 3.17. Asymmetry 2 of characters (1-4) of strains. Filled circles= regime FE, open circles= regime CE. N=16 families in each strain/regime.

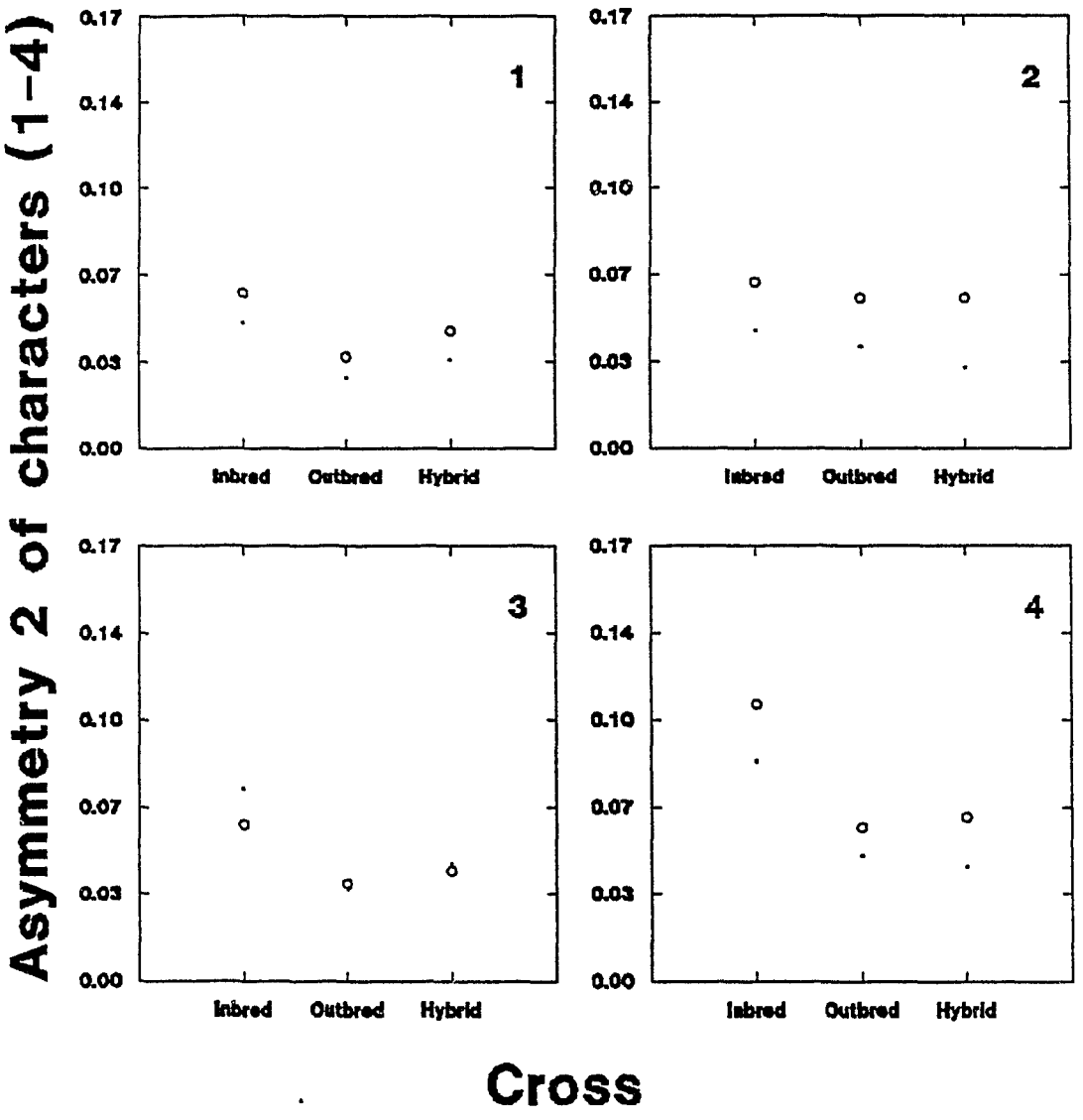


Fig. 3.18. Asymmetry 2 of characters (1-4) of crosses. Filled circles= regime FE, open circles= regime CE. N=16 families in each cross/regime.

4.3 SUMMARY of Method 2

Directional asymmetry and antisymmetry were relatively unimportant to the total asymmetry variation, validating that Asymmetry 2 values are a good estimate of FA. Asymmetries of all characters were significantly different between regimes. Asymmetries of characters (1), (2) and (4) were higher in regime CE than in regime FE. The largest difference between regimes was for character (2), corresponding to the Asymmetry 1 results. Lines were significantly different for all characters in both regimes. The overall ranking in regime FE was A>C>B>F>E>D, and for regime CE ranking was A>B=D=F>E>C. Nifi strain had the highest Asymmetry 2 values for all characters in both regimes. Inbreds had the highest level of Asymmetry in both regimes (due to influence of inbred line A).

5 Discussion

Comparison of Methods: Asymmetry 1 and 2

The correlations between Asymmetry 1 and 2 for each character are presented in Table 3.17. All correlations between methods of estimating FA are strong and significantly positive (Table 3.17). Correlations are stronger within regime FE than in CE. The results of the 2

methods of estimating FA are fairly similar; the discrepancies are discussed later in the text. The consistent results are:

1) Regime: The effect of regime was largest in character (2). The level of asymmetry was higher in regime CE than in regime FE for all characters (except for character (3) using method Asymmetry 2).

2) Line: Line A (inbred Nifi) had the highest level of asymmetry.

3) Strain: Nifi had higher levels of asymmetry than did Israel and Hybrids.

4) Cross: Inbred had higher level of asymmetry than did outbred or hybrids using Asymmetry 2, but the result was heavily influenced by inbred Nifi. There was no significant difference among crosses using Asymmetry 1.

The results common to both methods are certainly conclusive. Discrepancies arise because several factors were significant using Asymmetry 2 yet nonsignificant using Asymmetry 1. Asymmetry 1 method is a relatively pure estimate of FA (Palmer and Strobeck 1986) however, in the present study, the high level of variation among families within line and the low number of families within lines, decreased statistical power to detect significant differences among factors. For example, the mean squares

attributable to regime border on significance in characters (1) and (4) and was significant in character (2) using Asymmetry 1, but regime was significant in all characters using Asymmetry 2. The effect of line was only significant in character (1) using Asymmetry 1. However, because of the increased statistical power, lines were significantly differently in all characters using Asymmetry 2. Similarly, strain and cross had a significant effect in various charaters using Asymmetry 2 yet only strain was significant, in character (1), using Asymmetry 1.

The Asymmetry 1 method is considered to be the purest estimate as it isolates the FA component of variance from other sources. However, the results of Asymmetry 2 show that directional asymmetry and antisymmetry sources of variation were not important to the total variation, and therefore, Asymmetry 2 is a credible estimator of FA. This is confirmed by the strong correlation between Asymmetry 1 and Asymmetry 2 methods in all characters. The lack of significance in Asymmetry 1 analyses stems from high variation between families within lines and low degrees of freedom using family mean values. The Asymmetry 2 method uses individual instead of family values and is statistically more powerful.

An important point, when considering experimental design, is that the largest source of variation is among families for characters (1), (3) and (4) using the Asymmetry 1 method. Note that the variation accounted for by ANOVA

models was much greater when families were nested within line (compare Tables 3.7-3.9). Trexler and Travis (1990) investigated the genetic basis of fitness traits of the Sailfin molly (Poecilia latipinna) in different environments. They used a nested ANOVA design with family groups nested within population of origin. The largest source of variation was among families and a few outliers were detected. Indeed, when the authors repeated their experiment with different families, the results were not consistent in that they detected genotype*environment interaction in one experiment but not in another. The consistent result in the second experiment was that, again, variation among families was high. The authors attribute their results to the fact that even though populations were not overly different, they sampled average families but on occasion sampled genotypes from the extreme end of the population distributions. They concluded that the largest amount of fitness variation was among families. Both Trexler and Travis (1990) and my own results suggest that when families have a strong identity, experiments to test genotypes in different environments may not be repeatable.

With respect to experimental design, if the desire is to detect differences among populations and only family values are to be used (as when using Asymmetry 1 method) then the number of families/population should be maximized. Often, maximizing family number is impossible; my own

experiment was constrained by the number of available parents to create inbred strains and by rearing space. In addition, variation between replicates is particularily high in fish studies and appears not to be normally distributed (Uraiwan and Doyle 1986). High variation between families is the norm in fish studies and will only be suppressed statistically by using large numbers or by using alternate experimental designs (Basiao and Doyle 1990). In the present study, the strong family identity was not due to differences among tanks because each family was separated into 2 tanks, representing regime FE and CE. Therefore, the between family variance component was not caused by a tank effect.

Research is further constrained in fish that produce live young and where spawning is asynchronous. In circumstances where resources are limited or when between-family variation is known to be high, the Asymmetry 2 method is superior to Asymmetry 1 in that individual, instead of family, values are used in the statistical analyses.

In conclusion, Asymmetry 2 is employable when the largest source of asymmetry variation is FA. Asymmetry 1 is the best method if variation among family means is low and/or resources and space are effectively unlimited, as in Drosophila experiments.

Expected vs Observed Results

Regime

Under the assumptions explained in chapter 1, the expected results were that higher levels of asymmetry would be observed in the more stressful environment of regime FE and in genetically inferior (inbred) groups. The results for 3 out of 4 character in the analysis of regime is opposite to what was expected, yet within regimes, inbred Nifi had higher levels of asymmetry. Therefore, FA acts as expected within regimes but not between regimes. These results would occur if 1) highly asymmetric individuals in regime FE died before they could be measured or if 2) FA variation were dependent on size. Firstly, 0-3 individuals died in each cell of regime FE summing to 22 mortalities, 0-4 individuals died in each cell of regime CE summing to 21 mortalities, thus there were no numerical differences in mortality between regimes. Secondly, the variances of signed differences (Asymmetry 2) do not appear to increase with size. Asymmetry 1 values , in which the variation due to size is factored out, were also larger in regime FE but only significantly so in character (2). However, the size effect could influence the data at the level of regime because the fish in regime CE are absolutely larger than in regime FE. My final judgement was that although the data are somewhat influenced by size, the effect is not strong enough to warrant presenting size-corrected data. As was previously

stated, the problem of correcting for size is that larger individuals would have proportionally smaller variances, thus biasing the data.

In any event, the higher levels of asymmetry observed in the relatively benign regime CE of this study, seriously undermines the potential use of FA as a convenient indicator of dietary stress.

Inbreeding

While inbred Nifi had the highest levels of asymmetry, inbred Israel was not significantly different from outbred Israel. Outbred Nifi had higher levels of asymmetry in most characters, and grew more slowly, than outbred Israel strain. It may be that the effect of one generation of brother-sister mating did not have a detrimental effect on the Israel strain because they are a superior strain to begin with.

Hybrids

The effect of hybridization is dependent on the genetic divergence between parental strains. Theory states that hybridization can result in a decrease in FA, thus an increase in developmental stability (Leamy and Atchley 1985; Palmer and Strobeck 1986). As the level of differentiation increases, there will be a point at which a decrease in FA reverses due to divergent parental genomic combinations

(Vrijenhoek and Lerman 1982). As there is no previous knowledge of the genetic divergence between Israel and Nifi strains, there was no expected result except that the hybrids are presumably more heterozygous than either parental strain. The hybrids of this study did not indicate that Nifi and Israel strains are genomically incompatible. In general, hybrid levels of asymmetry were not as high as the Nifi strain suggesting that the Nifi strain might even be improved by introgression of Israeli genes.

Genotype/environment Interaction

There are many reports that as stress increases, the fitness of genetically inferior organisms decreases most rapidly, and that the difference between inferior and superior genotypes is larger in stressful environments (Parsons 1990c). A corollary prediction is that if inbreds are more susceptible to environmental stress, then as stress increases, the FA of inbreds will increase at a faster rate than outbreds or hybrids. Statistically, the similarity of genotype response to the level of stress is typically measured by the magnitude of genotype (family, line or strain or cross)*environment (regime) interaction term; a significant genotype*environment interaction term indicates that genotype ranking is dependent on the level of environment. Genotypes that have the same value in both

environments are unresponsive, genotypes with very different values are responsive (termed susceptible if the change in value is non-adaptive).

There were no significant interactions between regimes and genetic composition (line, strain, cross) as measured by Asymmetry 1 due to the low mean squares ascribed to both factors and the high variation among families. A family* regime interaction could not be tested due to insufficient degrees of freedom. However, families, lines, strain and crosses do change rank between regimes in some characters. In order to determine the extent of similarity between a given family in regime FE and the same family in regime CE, the correlation of families between regimes was calculated. This correlation is a measure of the genotype*environment interaction (Via 1987). Although, note that a correlation represents relative ranking whereas a genotype*environment interaction represents the response to different environments. Therefore, genotype ranking can remain the same across environments while genotype responses to different environments can differ significantly (de Jong 1990). Using Asymmetry 1, the family correlations between regimes are: character(1) $r=0.525$ ($df=20$, $p<0.05$), character(2) $r=0.045$($df=20$, p=ns), character(3) $r=0.268$($df=20$, p=ns), character(4) $r=0.567$ ($df=20$, $p<0.01$), average Asymmetry 1 $r=0.533$ ($df=24$,$p<0.01$).

The similarity between family values is strong in characters (1), (4) and average Asymmetry 1, another indication of a strong family component. The nonsignificant correlation in character (3) is due to the low variation of FA values in both regimes. The nonsignificant correlation of character (2) indicates a change in family ranking dependent on regime.

Correlation among characters (1-4)

In general, the asymmetries of characters (1) (2) and (4) are weakly correlated. Character (3) does not correlate well with other characters because it itself does not vary. The weak correlations can be be due to several factors: a small range of asymmetry values, or the difference in function of the morphological character. If a bilateral character has an important function requiring coordination, for example locomotion or dexterity, then natural selection selects for precise development and the result will be little or no asymmetry (Leamy and Atchley 1985). However, as Lamb et al (1990) point out, many researchers have found significant FA differences using cranial characters. Although the notion that selection for symmetry in functional morphological characters is logical, it is difficult to test without invoking tautological adaptive hypotheses. By way of a successful study, Alexander et al (1984) studied the symmmetry of the mechanical properties of limb bones in

birds. They assumed variability between homologous bones on one individual to be a reflection of error in an optimal (thus most economical) construction. The authors found that bone strength of wild Lesser Black-backed gulls (Larus fuscus) showed a greater symmetry of bone strength (maximum load before fracture) and work (amount of work required to break the bone) than in domestic birds (Gallus gallus). They concluded that selection for symmetry is more important in wild than in domesticated species. In this study, it would be hard to argue that all 4 characters were constrained by selection. It cannot be explicitly stated that the FA characters were influenced by selection on trait symmetry.

Comparison of Morphological Characters to measure FA

Character (1) is the best indicator of genetic differences and it is negatively correlated with growth.

Character (2) is the best indicator of regimes yet a poor indicator of genetic differences.

Character (3) shows no difference among types or correlation with growth. The range of values of character (3) is relatively small rendering it an uninterpretable and therefore a useless measure of FA.

Character (4) borders on significance for both regime and genetic composition.

With respect to a composite index, it would be best to construct one using characters (1), (2) and (4).

Conclusion

The results show that asymmetry was higher in regime CE than in regime FE, highest in the Nifi strain and in inbred Nifi, and that asymmetry of character (1) was negatively correlated with growth. In this conclusion I address the practicality of FA as an indicator of genetic or environmental stress in tilapia populations.

In controlled laboratory conditions, an increase in FA has been frequently associated with genetic stress (for example, directional selection, inbreeding or hybridization between genetically divergent genomes) and with enviromental stress (where severity can be determined and controlled). In field conditions, the published results are more equivocal. Genetic histories are better documented in laboratory populations of Drosophila and mice than in wild or recently domesticated species. Environmental stress can be determined and regulated in the lab, but environmental stress in the field is defined only by its relative severity (Parsons 1990c). That is, an increase in FA with genetic and environmental stress may not always be detected in the field as the relationship is dependent on the underlying genetic history of a population and its perception of environmental stress.

In my study, the genetic background of both strains is not completely known, yet the results do show an effect of strain and inbreeding. FA has potential as an indicator of genetic stress, character (1) being the best candidate to do so. Given that protein is of utmost importance to tilapia (Bowen 1982), I consider regime FE to be a fairly severe environmental stress and that further stress would have resulted in some mortalities. I am not certain why the effect of diet was the opposite of what was expected in 3 out of 4 characters, and can only conclude that the relationship between FA and dietary stress in tilapia has yet to be resolved. Ultimately, an indicator of sublethal stress is desirable in aquaculture systems; given the practical and statistical rigor required to measure FA and that the genetic and environmental differences were marginal, FA is more suitable to evolutionary questions in the lab than it is to practical management procedures on farms.

TABLE 3.17. Spearman correlation coefficients among Asymmetry 1 and 2 of pooled (N=48) and within regimes (N=24): Asym 1*Asym 2=correlation between Asymmetry 1 and Asymmetry 2. All correlations are significant at $p<0.001$ or $p<0.01$.

	Asym 1*Asym 2		
	POOLED	Regime FE	Regime CE
Character			
(1)	0.879	0.823	0.964
(2)	0.807	0.941	0.611
(3)	0.873	0.939	0.773
(4)	0.778	0.919	0.656
Average	0.916	0.940	0.876

Chapter 4. Scale Morphology

1 Abstract

Growth and the stabilities of growth and development were examined in inbred, outbred and inter-strain hybrid lines of tilapia (Oreochromus niloticus) under 2 diet regimes. Developmental stability was quantified as the inverse of the number of deformed circuli (calcified ridges on the scales). Growth stability was measured as the inverse of the individual variability of scale circulus spacing (circulus spacing is an indirect measure of growth rate). Families from each line were fed an alternating diet of rice bran and commercial pellet, or a constant diet of commercial pellets. The difference in regimes had a significant effect on growth, developmental stability and growth stability. Genetic composition of line had a significant effect only on developmental stability. Inbred lines had lower developmental stability than outbred lines. The "Nifi" strain had lower developmental stability than the "Israeli" strain, hybrid progeny of the 2 strains had higher developmental stability than the average of the parent strain values. At the individual level, faster growing fish had higher developmental and growth stabilities. At the family level, faster growing families had lower developmental stability and higher variability of growth,

but only within the constant nutritional environment. Composite indices of merit can be constructed by summing the variables, weighted according to the correlations among variables and breeding objectives. Composite indices have applications as indicators of genetic and environmental stress and as a selection index.

2 Introduction

The previous chapter described developmental stability as measured by FA. This chapter describes developmental stability and an additional variable, growth stability, as measured by scale morphology. As was argued in Chapter 1, if a breeder's objective is to develop fish that are capable of good, reliable growth in fluctuating ecosystems, then the criterion of "merit" in the selection program should incorporate both growth and developmental stability (stress resistance). In this chapter, I analyse an additional variable, growth stability, and include it with growth and developmental stability in an index of overall merit of individual tilapia. I also examine the relationships among growth, developmental and growth stabilities, in the context of their implications for aquaculture bioeconomics and the evolutionary genetics of tilapia. As detailed in the following sections, developmental stability is quantified as the inverse of the number of deformed circuli (calcified ridges on the scales) per individual. Growth stability (ability to maintain constant growth rate during environmental fluctuations) is measured as the inverse of individual variability of spacing between circuli (circulus spacing is an indirect measure of growth rate (van Oosten 1957; Doyle et al 1987).

Both the economic and the natural, or evolutionary strategies of genetic improvement can involve combinations of fast or slow growth, and high or low developmental and growth stabilities. The preferred bioeconomic strategy is dependent on the underlying genetic correlations and on breeding objectives. A composite index of merit (growth, developmental stability and growth stability) embodies a bioeconomic strategy and has application as an indicator of genetic and environmental stress and as a selection index. In this chapter, I present an example of a hypothetical composite index of merit by summing the variables, weighted according to the correlations among variables and a provisional set of breeding objectives. The provisional bioeconomic assumption of this chapter is that fast growth, high developmental and growth stabilities are desirable traits in aquaculture systems in developing countries. This is not a universal strategy but the example serves to demonstrate how the 3 criteria can be combined.

Circulus deformation

The literature review in this chapter is short because, to my knowledge, scale circulus deformations have not yet been used as an index of developmental stability.

The structure of the anterior, or growing, edge of the scale does not change after it has formed (Sire 1986) so the scale serves as a record of past development and growth.

Scale growth has repeatedly been used as an estimate of change in fish length (Jensen 1957; Ottoway and Simkiss 1977; Ottoway 1978; Payne and Collinson 1983; Doyle et al. 1987; Weatherly and Gill 1987 and references therein; Barber and Walker 1988; Galloway 1988) but additional scale characteristics involving its formation and structure have not been as thoroughly researched. It is worth noting that Lippitsch (1990) has recently completed a thorough characterization of cichlid scale morphology and squamation patterns for use in systematics.

As early as 1930, Gray and Setna reported that circuli were deformed in semi-starved fish as compared to fish fed to satiation. Reproduction and cold temperatures also cause the formation of irregular circuli and are often referred to as 'checks' in the literature. Fagade (1974, c.f. Weatherly and Gill 1987) observed altered formation of circuli in a temperate fish, corresponding to a transfer from brackish to fresh water. Circuli, ossified ridges, are deposited as the scale grows. Circuli usually appear as crescent shaped ridges, evenly spaced along a sector. When a fish is stressed, the uniformity of circulus formation is disrupted and/or the even spacing between circuli becomes irregular. These two phenomena were used to quantify abnormal development of the scale.

The amount of deformed circuli, as an inverse index of developmental stability, rests on the following assumption. It is assumed that repeated structures are formed through the activity of the same genes and that an abnormal structure results because the expression of genes was affected by internal and/or external environments (Falconer 1989). Falconer (1989) refers to a decrease in repeatability of developmental events as a measure of developmental noise. For example, Reeves (c.f. Falconer 1989) studied the repeatability of abdominal bristles on Drosophila melanogaster. Reeves used the amount of variation between repeated events of bristle formation as a measure of developmental variation arising from the environment. In my case, circulus deposition on scales is a repeated developmental event; deformations are an indication of developmental noise. When tilapia undergo an environmental change, for example transfer from hatchery to fish pond, or from fresh to saline water, scale circuli are often laid down abnormally, e.g. broken or fused or incomplete. I take this to be an indication of stress resulting from the change, or subsequent to it. The type of stress is not recorded on the scale but the severity of stress can be measured by the amount of circulus deformation. In this study, the abnormality of circulus formation was quantified and used as an inverse index of developmental stability across environments. The additional variable, individual

variability of circulus spacing, was measured as an inverse index of growth stability. Growth stability was measured chiefly in order to determine its relationship with developmental stability and growth.

3 Material and Methods

Growth measure

Standard length, measured at 0 and 6 weeks, was used to estimate growth rate directly. Percent specific growth rate/day was estimated as:
((Individual log standard length at week 6 - family mean log standard length at week 0)/number of days) * 100).
The growth measure of this chapter differs from previous chapters in that individual estimates are used. Note that individual starting lengths were not obtained so the growth estimates of individual animals are subject to some error.

Scale measurements:

Figure 4.1 shows a tilapia scale. The anterior portion is the growing edge which is divided into 2-10 individual sectors. Circuli, ossified ridges, are deposited as the scale grows. The usual morphology of a circulus is as a

crescent shaped ridge, evenly spaced along a radius. When a fish is stressed, the uniformity of circulus formation is disrupted and/or the even spacing between circuli spacing becomes irregular.

Developmental Stability

Circulus deformation was used as an inverse measure of developmental stability and is referred to as "deformation score". The various types of deformation were categorized and are labelled in Fig 4.1. Deformations were scored on the innermost 5 sectors; the central sector, and the 2 sectors on each side of the central sector. The magnitude of deformation was measured by scoring the total number of deformed circuli on each scale. Data were log-transformed prior to analysis to minimize the effect of scale size and to equalize variances between regimes.

Growth Stability: Individual variability of growth

The measure of growth stability is referred to as "individual variability of growth". Fish with relatively wider spacing between circuli have higher growth rates (Doyle et al. 1987). When the rate of growth is decreased, the spacing between circuli is either reduced or circuli are not completely laid down (Bilton and Robins 1971a,b). Similarly, when conditions allow for an increase in growth, circulus spacing becomes wider. Individual variability of

growth was measured as follows: Circulus spacing was measured at 7 radial distances along the central sector of the scale. Individual variability of growth was estimated simply by calculating the variance of 7 circulus spacings on each individual. Data were log-transformed prior to analysis due to underlying chi-squared distribution of variances and to equalize variances between regimes.

Scales were wet-mounted on glass slides and measured on a Leitz Projection microscope at 100x. Two scales per fish, 15 fish per each regime/line/family combination were measured. The average of the 2 measurements per fish was used in the subsequent analyses.

Composite Index of Overall Merit

A composite index of individual merit was constructed based on my operational definition of merit; fast growth, high developmental and growth stabilities. Deformation score is entered inversely in the index, as it is a measure of the loss of integrity of development and a decrease in developmental stability. Individual variability of growth is entered inversely as it means that the growth of a genotype is relatively sensitive to environmental fluctuations. Accordingly, a high merit value represents a relatively fast growing fish, with high developmental stability (low deformation score) and a stable pattern of growth (low individual variability of growth). Since there is as yet no

empirical information on the relative economic value of the component variables, the component variables were standardized so they contribute equally to the variance of the index. Ideally, the components of merit indices used in selection programs should be weighted by heritabilities and relative economic values (Falconer 1989,p.326). The index is merely designed to demonstrate how 3 important criteria can be combined to assess overall merit. The index was calculated using the following equation:

MERIT=standardized growth - standardized deformation score - standardized individual variability of growth

In particular, I was curious as to whether growth is positively or negatively correlated with the other two index components. If genetic correlations are positive, it signifies potential trouble for stock improvement programs. It is commonly found that high yield is positively correlated with sensitivity in domesticated crops (Jinks and Pooni 1988). It should be noted that in this study it was clear that individual variability of growth would be higher in regime FE because the regime was changed from rice bran to commercial food pellet for an intermediate period of 2 weeks and then shifted back to rice bran. The CE group was fed a constant diet of commercial food pellet. The mean

variability of growth was naturally higher in regime FE because individuals had to adjust to the change in type of diet.

Statistical analyses

Regime and Line

A 2-way ANOVA with sampling was used for the main analysis of four variables, growth, deformation score, individual variability of growth and standardized composite merit. The factors are regime and line, and families are nested within line.

$$Y_{ijkl}=\mu + R_i + L_j + F_{k(j)} + E_{ijkl}$$

Regime, strain and cross

The overall effects of strain and cross effects were analysed in simple 3-way ANOVAs.

$$Y_{ijkl}=\mu + R_i + Strain_j + Cross_k + E_{ijkl}$$

Where Y is individual observation of one of the 4 variables (growth, deformation score, individual variability of growth, merit); l is individual; μ is the population mean; R is the effect of i^{th} regime (2 levels); L is the effect of

the j^{th} line (6 levels); F is the effect of the k^{th} family (4 families) nested within the j^{th} line and E is the random error term of individual fish (average 16/cell). Strain is the effect of the j^{th} strain; Cross is the effect of the k^{th} cross. Interaction terms were included in the models.

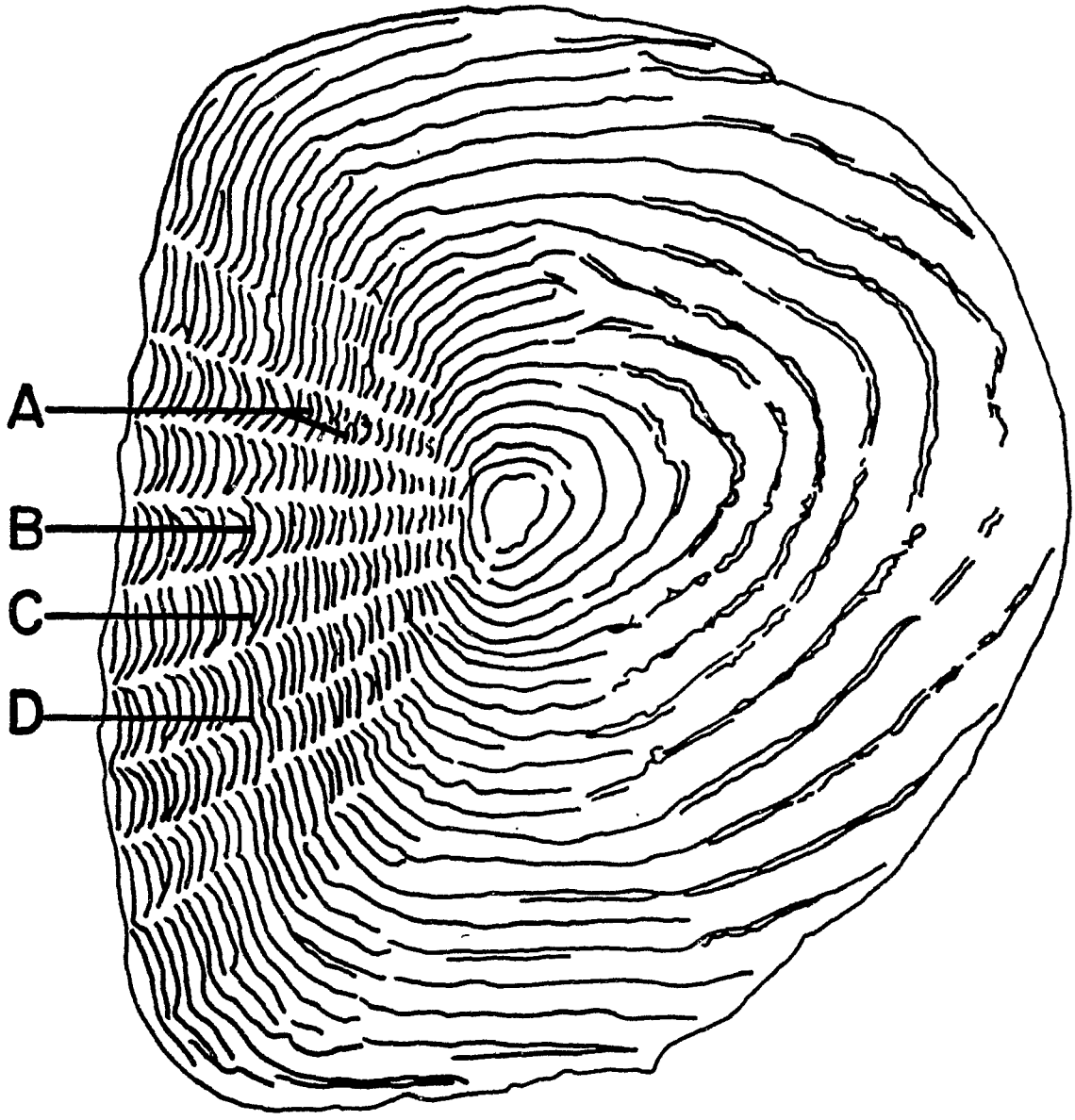


Fig. 4.1. Tilapia scale showing deformed circuli. Anterior margin (left-hand side) is growing edge. Types of deformation are labelled: A) deposited incompletely B) fused with adjacent circuli C) overextended into the inter-sector space D) extended into the next sector to form a band across the anterior portion of the scale or E) irregular deposition resulting in a partially inverted circuli; very similar to type A (E is not shown).

4 Results

4.1 Homogeneity of Regime Variances

The untransformed variances for deformation score and individual variability of growth were much greater in regime FE than in CE. Log-transformation equalizes the variance between regimes for both variables. Note that the means of both deformation score and individual variability of growth were greater in regime FE than in regime CE as expected (Table 4.1).

TABLE 4.1. Regime means and standard deviations (individuals pooled within regime) of original and log-transformed data deformation score and individual variability of growth values (+/- standard deviation, in parentheses).

	Original Data	
	Deformation Score	Individual variability of growth
REGIME FE		
Mean (S.D.)	20.87 (11.93)	3.45 (2.88)
REGIME CE		
Mean (S.D.)	9.43 (6.84)	2.18 (1.91)
[1]Chi-square	111.426	62.619
Probability	0.000	0.000

	_LOG-Transformed Data	
	Deformation Score	Individual variability of growth
REGIME FE		
Mean (S.D.)	1.25 (0.28)	0.39 (0.39)
REGIME CE		
Mean (S.D.)	0.89 (0.28)	0.20 (0.37)
[1]Chi-square	0.088	0.848
Probability	0.767	0.357

[1]Bartlett's Test for Homogeneity of Group Variances with 1 df. Probability denotes probability that group variances are significantly different.

4.2 ANOVAs

Effect of Regime and Line

The effects of regime and family nested within line were significant in all 4 analyses. The effect of line was significant in the analyses of deformation score and composite merit, but nonsignificant in the analysis of growth and individual variability of growth (Table 4.2, Fig. 4.2). The effect of line in the analysis of deformation score was significant, although small relative to the effect of regime. The significant effects of regime and line were further analysed with an interaction term in the ANOVA to see if the response of lines was similar at each level of regime. The interaction term was nonsignificant, indicating that lines responded predictably to level of dietary regime. However, when variables were analysed in a 2-factor with regime (2 regimes) and family (24 families) as factors, the interaction term of regime*family was significant. The results are examined thoroughly below, after presentation of main effects.

Overall, the mean deformation scores of the inbred Nifi and Israel lines were 6.26 % and 15.45% higher than the corresponding outbred lines. The means of the hybrid Israel X Nifi and Nifi X Israel were 5.93% and 4.40% less than the average of outbred Nifi and Israel. The line mean score of outbred Nifi was 18.37% higher than outbred Israel (Table 4.3).

Effect of Strain and Cross

To determine the effect of inbreeding, hybrids were dropped from the analysis and lines were re-categorized according to level of inbreeding (cross) and their strain of origin (strain). Data were analysed using ANOVA with 3 factors, regime, strain and cross. The effect of regime is significant in analyses of all 4 variables. The effect of cross and strain were significant in the analysis of all variables except individual variability of growth (Table 4.4, Fig.4.3, Fig.4.4). The interaction terms indicate that inbred groups have a similar response to level of dietary regime, that strains respond dissimilarly with respect to deformation scores to level of dietary regime and that the growth response of inbred groups is dependent on strain. In general, inbreds grew 3.5% less, had 10.42% higher deformation scores and 5.88% less individual variability of growth than outbreds. The Nifi strain grew 13.07% less, had 13.32 % higher deformation scores and 22.40% less individual variability of growth than the Israel strain (Table 4.5).

TABLE 4.2. ANOVAs of 4 variables (growth, deformation score, individual variability of growth and composite merit) with 2 factors, Regime (2 levels) and Line (6 levels) with families (4 families): N=760; df=degrees of freedom, FwL=family nested within line, MS=mean square, p=probability of significance.

		Growth R^2=0.580			Deformation Score R^2=0.428		
	DF	MS	F	P	MS	F	P
Regime	1	5.761	389.728	***	24.380	376.869	***
Line	5	0.743	2.307	ns	1.401	5.388	**
FwL	18	0.322	21.788	***	0.260	4.014	***
Error	735	0.015			0.065		
		Variability of Growth R^2=0.176			Composite Merit R^2=0.501		
	DF	MS	F	P	MS	F	P
Regime	1	7.118	55.462	***	1210.452	551.454	***
Line	5	0.847	1.870	ns	45.538	4.746	**
FwL	18	0.453	3.527	***	10.438	4.755	***
Error	735	0.128			2.195		

n.s. not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 4.3: Line means of pooled regimes (n=8 families) ((+/-) standard deviation in parentheses). Variable codes are: (I) growth (II) deformation score (III) individual variability of growth and (IV) composite index of merit.

	I	II	III	IV
Inbred Nifi	0.727	1.205	0.181	-0.324
	(0.213)	(0.280)	(0.360)	(1.845)
Outbred Nifi	0.650	1.134	0.252	-0.707
	(0.156)	(0.266)	(0.263)	(1.671)
Inbred Israel	0.727	1.106	0.299	-0.331
	(0.170)	(0.361)	(0.428)	(2.317)
Outbred Israel	0.857	0.958	0.259	0.926
	(0.166)	(0.342)	(0.453)	(2.174)
Hybrid I X N	0.789	0.984	0.433	0.033
	(0.150)	(0.299)	(0.360)	(1.896)
Hybrid N X I	0.320	1.000	0.346	0.377
	(0.168)	(0.355)	(0.392)	(2.013)

TABLE 4.4. ANOVAs of 4 variables (growth, deformation score, individual variability of growth and composite merit) with 3 factors, Regime(R) (2 levels), Cross(C) (2 levels; inbred or outbred) and Strain(S) (2 levels; Nifi or Israel): N=511; R*C, R*S,C*S=interaction terms; df=degrees of freedom, MS=Mean squares from ANOVAs, P=probability.

		Growth R^2=0.355			Deformation Score R^2=0.398		
	DF	MS	F	P	MS	F	P
Regime	1	3.862	160.114	***	16.840	258.937	***
Cross	1	0.122	5.076	*	1.819	27.970	***
Strain	1	1.464	60.700	***	2.691	41.374	***
R*C	1	0.013	0.554	ns	0.068	1.045	ns
R*S	1	0.008	0.314	ns	0.517	7.951	**
C*S	1	1.410	58.438	***	0.237	3.639	ns
Error	504	0.024			0.065		
		Variability of Growth R^2=0.084			Composite Merit R^2=0.457		
	DF	MS	F	P	MS	F	P
Regime	1	4.901	35.648	***	826.308	338.865	***
Cross	1	0.012	0.085	ns	32.419	13.295	***
Strain	1	0.434	3.154	ns	96.317	39.499	***
R*C	1	0.002	0.017	ns	0.001	0.001	ns
R*S	1	0.478	3.477	ns	12.129	4.974	*
C*S	1	0.450	3.271	ns	92.651	37.996	***
Error	504	0.137			2.438		

n.s. not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

TABLE 4.5. Strain means of pooled regimes (n=16) ((+/-) standard deviation in parentheses). Variable codes are: (I) growth (II) deformation score (III) individual variability of growth and (IV) composite index of merit.

	I	II	III	IV
Nifi	0.689	1.170	0.215	-0.511
	(0.191)	(0.275)	(0.318)	(1.769)
Israel	0.793	1.031	0.279	0.305
	(0.180)	(0.358)	(0.440)	(2.328)
Hybrids	0.805	0.992	0.389	0.207
	(0.160)	(0.328)	(0.378)	(1.960)

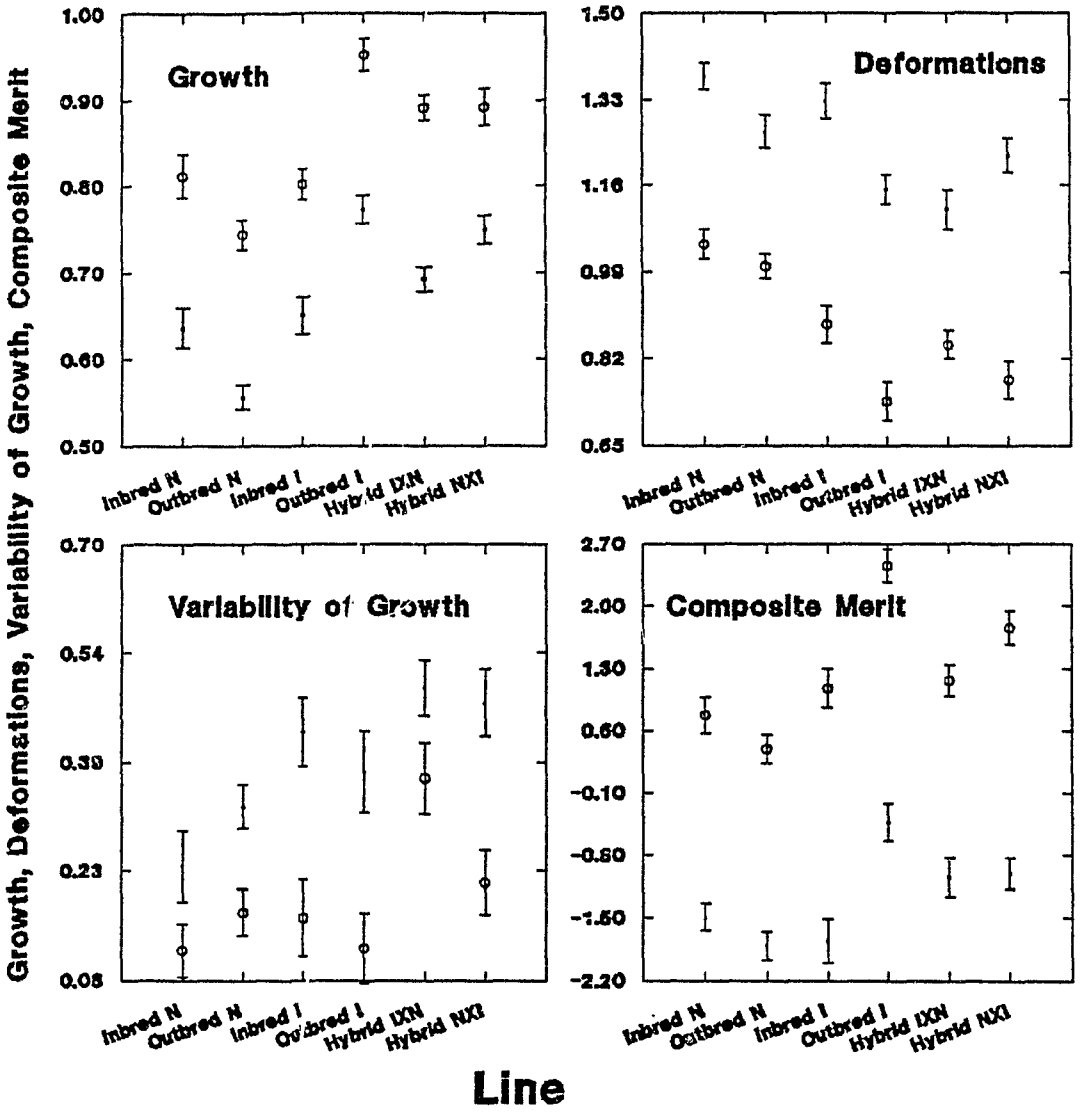


Fig. 4.2. Growth, Deformation Score, Indivdual Variability of Growth and Composite merit (sum of growth, inverse number of deformed circuli and inverse variability of growth) plotted against line. Each point is mean score of 4 families per line, within regime. Error bars are standard error of the mean. I=Israel N=Nifi, Filled circles= regime FE, open circles= regime CE.

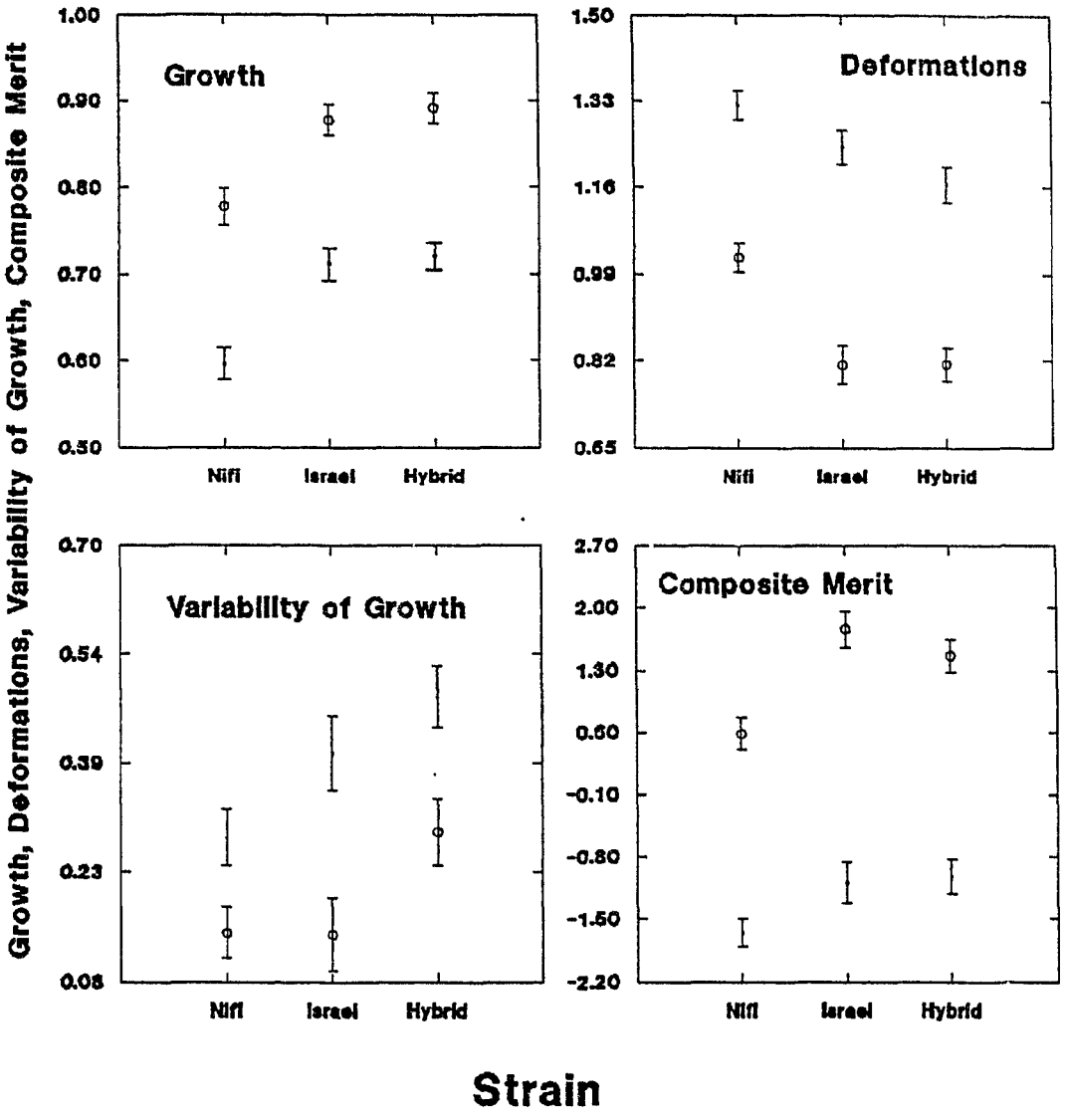


Fig. 4.3. Growth, Deformation Score, Indivdual Variability of Growth and Composite merit (sum of growth, inverse number of deformed circuli and inverse variability of growth) plotted against Strain. Each point is mean score of 8 families per strain, within regime. Error bars are standard error of the mean. Filled circles= regime FE, open circles= regime CE.

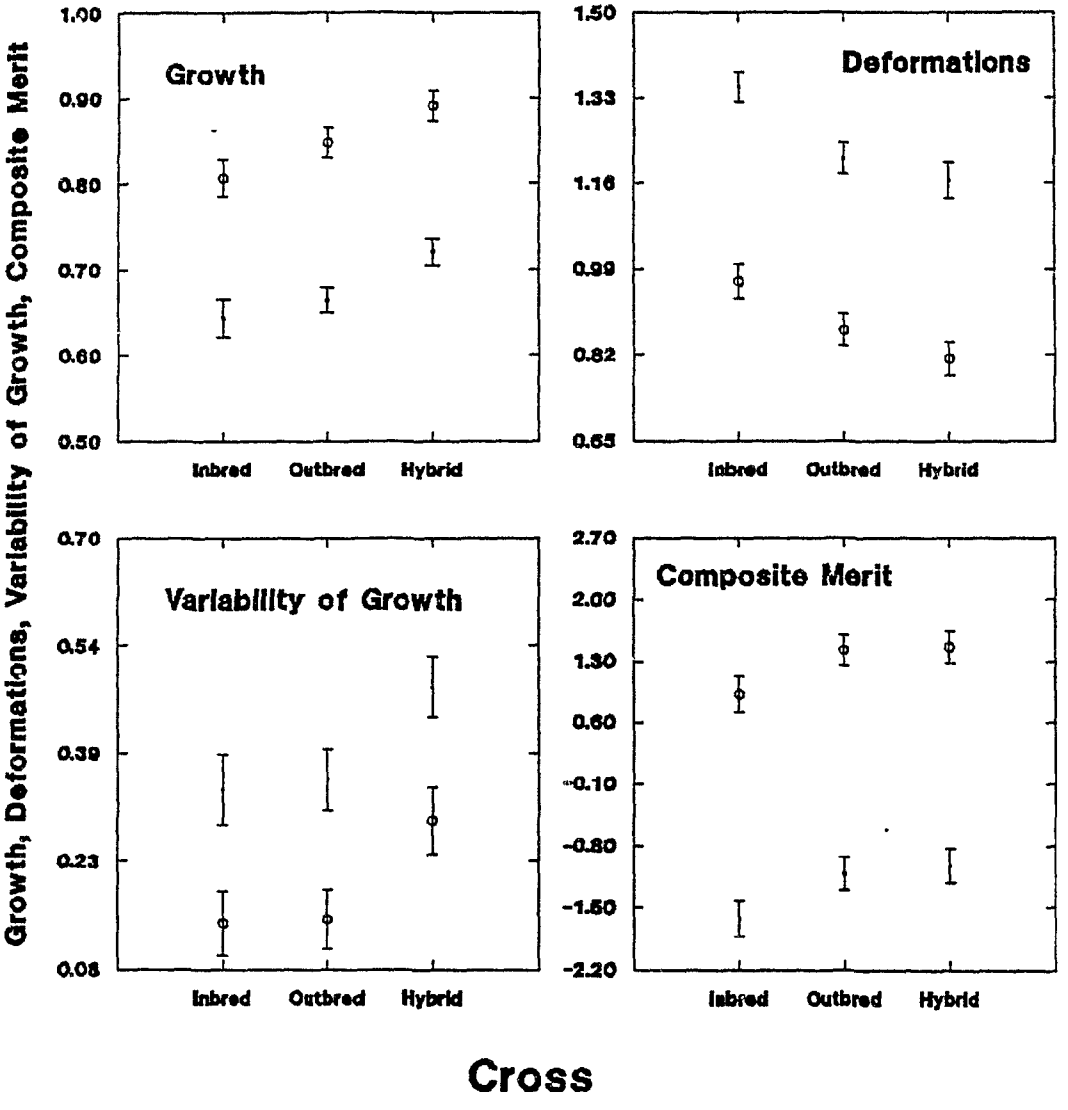


Fig. 4.4. Growth, Deformation Score, Indivdual Variability of Growth and Composite merit (sum of growth, inverse number of deformed circuli and inverse variability of growth) plotted against Cross. Each point is mean score of 8 families per line, within regime. Error bars are standard error of the mean. Filled circles= regime FE, open circles= regime CE.

4.3 Regime*Family Interaction

The analysis of regime and line indicated a strong family component, as in chapter 3. To investigate this further, families were analysed in an ANOVA with 2 factors, regime and family. A regime*family interaction term was included in the model to determine the extent of similarity of family response to level of regime. In all variables but growth, the interaction term is significant. Families which have low deformation scores or individual variability of growth in one regime do not necessarily have low values in the other (Table 4.6).

TABLE 4.6. ANOVAs of 4 variables (growth, deformation score, individual variability of growth and composite merit) with 2 factors, Regime (2 levels) and Family (24 levels): N=760; df=degrees of freedom, R*F=Regime * Family interaction term, MS=mean square, p=probability of significance.

		Growth R^2=0.598			Deformation Score R^2=0.516		
	DF	MS	F	P	MS	F	P
Regime	1	5.722	391.816	***	24.361	431.525	***
Family	23	0.414	28.360	***	0.500	8.864	***
R*F	23	0.020	1.391	ns	0.320	5.664	***
Error	712	0.015			0.056		

		Variability of Growth R^2=0.245			Composite Merit R^2=0.551		
	DF	MS	F	P	MS	F	P
Regime	1	6.992	57.625	***	1220.742	589.708	***
Family	23	0.567	4.675	***	18.645	9.142	***
R*F	23	0.345	2.843	***	7.008	3.436	***
Error	712	0.121			2.040		

ns= not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

4.4 Correlation at individual level: within groups

Regime FE is, on average, a more stressful macro-environment than is regime CE. Within each regime every container is a separate environment. This is particularily true for fish that might grow in accordance to position in a social hierarchy, which defines the micro-environment. Consequently, individual response is dependent on each social environment, in this study each of 48 regime/line/family combinations represents a separate environment. Correlations among growth, deformation score

and individual variability of growth were calculated within each of the 48 combinations, i.e. each container (Table 4.7).

The underlying distribution of these correlation coefficients is unknown, so that the mean of several coefficients is uninterpretable using parametric statistics. However, we can conservatively assume that under a null hypothesis of zero true correlation, random observed correlation in the positive and negative directions are equally probable. I tested the significance of within-container correlations by testing if the observed frequency of negative and positive correlations that comprise the average differ from an expected ratio of 1:1. Goodness of Fit of single classification frequency distributions (Sokal and Rohlf 1981) was used to test the following correlations: (a) deformation score and individual variability of growth, (b) deformation score and growth and (c) individual variability of growth and growth. (Table 4.8). The G values (adjusted for sample size less than 200) for each relationship (a), (b) and (c) do differ significantly from our null hypothesis of a 1:1 ratio. Overall, relationship (a) is positive, (b) and (c) are negative. It can be concluded that individual fish with high deformation scores had less stable patterns of growth and grew more slowly than other fish in the same container.

TABLE 4.7. Individual level: Pearson product moment correlations within each family (1-4) within regime.(n=average 16 individuals per regime/line/family combination). Correlation coefficients are between the following variables:
(a) deformation score and individual variability of growth
(b) deformation score and growth
(c) growth and individual variability of growth

	_REGIME FE		
	(a)	(b)	(c)
Inbred Nifi			
1	-0.328	-0.258	0.604
2	0.425	0.120	-0.445
3	0.172	-0.348	0.534
4	0.079	-0.042	0.599
Outbred Nifi			
1	0.390	-0.633	-0.122
2	0.056	-0.423	-0.426
3	0.212	-0.009	-0.047
4	-0.114	-0.359	0.086
Inbred Israel			
1	0.337	-0.339	-0.272
2	0.138	-0.010	0.177
3	0.168	-0.618	0.016
4	0.687	-0.266	-0.473
Outbred Israel			
1	0.118	-0.102	-0.117
2	0.494	-0.271	0.191
3	-0.072	-0.447	0.316
4	0.340	-0.018	-0.118
Hybrid I X N			
1	0.697	-0.363	-0.259
2	0.221	0.070	0.000
3	0.385	-0.256	-0.188
4	0.079	-0.395	-0.205
Hybrid N X I			
1	-0.137	-0.134	-0.315
2	0.311	0.314	-0.328
3	0.055	0.487	-0.148
4	-0.629	0.374	-0.115

Table 4.7 (cont'd)

	REGIME CE		
	(a)	(b)	(c)
Inbred Nifi			
1	0.282	-0.310	-0.078
2	-0.457	-0.172	-0.047
3	-0.257	-0.172	-0.022
4	0.610	-0.354	-0.220
Outbred Nifi			
1	0.350	-0.530	0.243
2	0.043	0.190	-0.009
3	0.249	0.197	-0.050
4	0.318	-0.200	0.346
Inbred Israel			
1	0.004	0.262	0.077
2	0.216	0.162	0.029
3	0.525	0.295	0.261
4	0.515	-0.144	-0.342
Outbred Israel			
1	0.199	-0.298	-0.151
2	0.198	0.550	0.165
3	0.335	0.091	0.174
4	0.107	0.177	-0.042
Hybrid I X N			
1	0.077	-0.032	-0.320
2	0.136	0.187	0.231
3	-0.162	0.342	-0.657
4	0.166	-0.008	-0.270
Hybrid N X I			
1	-0.070	0.004	-0.041
2	-0.353	-0.265	0.721
3	0.195	-0.316	-0.128
4	-0.256	0.427	-0.061

TABLE 4.8. G-Test to test if the frequency ratio of the signs of observed data of (a), (b) and (c) are significantly different from the expected ratio of 1:1.

Sign of Correlations		Observed	Expected	G (adjusted)
(a)	-	11	24	14.72***
	+	37	24	
(b)	-	32	24	5.38*
	+	16	24	
(c)	-	31	24	4.14*
	+	17	24	

$Chi\text{-}square_{,0.001[1]}=10.827$; $Chi\text{-}square_{,0.01[1]}=6.635$; $Chi\text{-}square_{,0.05[1]}=3.841$

4.5 Correlation at the family level: among groups

The only significant correlations were within regime CE in which the correlation between deformation score and growth was significantly negative and the correlation between growth and individuality of growth was significantly positive (Table 4.9, Fig.4.5). If some fish do not grow at all, as was possible in regime FE, circuli will not be laid down (Bilton and Robins 1971a,b) deformed or not. It is possible that regime FE suppressed growth sufficiently so that some fish did not grow, the average deformation score is artificially low and thus the relationship between growth

and deformation score is obscured. Alternatively the relationship could not exist because scales of all fish were affected by regime FE despite differences in growth. This emphasizes the importance of environment when establishing correlations among variables.

Growth at the family level was positively correlated with variability of growth whereas at the individual level, they were negatively correlated. It cannot be assumed that the (macro) environmental differences observed at the family level in a single dietary regime were greater than the (micro) environmental differences experienced by individuals grown together within families. However, the relevance to genetic improvement programs is that families or strains are used to test performance across a range of environments, but individuals do not experience more than one macro-environment. The result was that the sign of the correlations at the family level apply only when dealing with discrete macro-environments.

In summary, on average, families which grew faster had a less stable pattern of growth but within each family, individuals with higher growth rates had a more stable pattern of growth.

TABLE 4.9. Family Level: Pearson product moment correlation using family means within regime.(n=24 families within each regime; n=48 when regimes are pooled). Correlation coefficients are between the following variables:
(a) deformation score and individual variability of growth
(b) deformation score and growth
(c) growth and individual variability of growth

	(a)	(b)	(c)
REGIME FE	0.105	-0.130	0.369
REGIME CE	-0.094	-0.586**	0.470*

n.s. not significant; * Significant at 0.05 P level; ** Significant at 0.01 P level; ***Significant at 0.001 P level

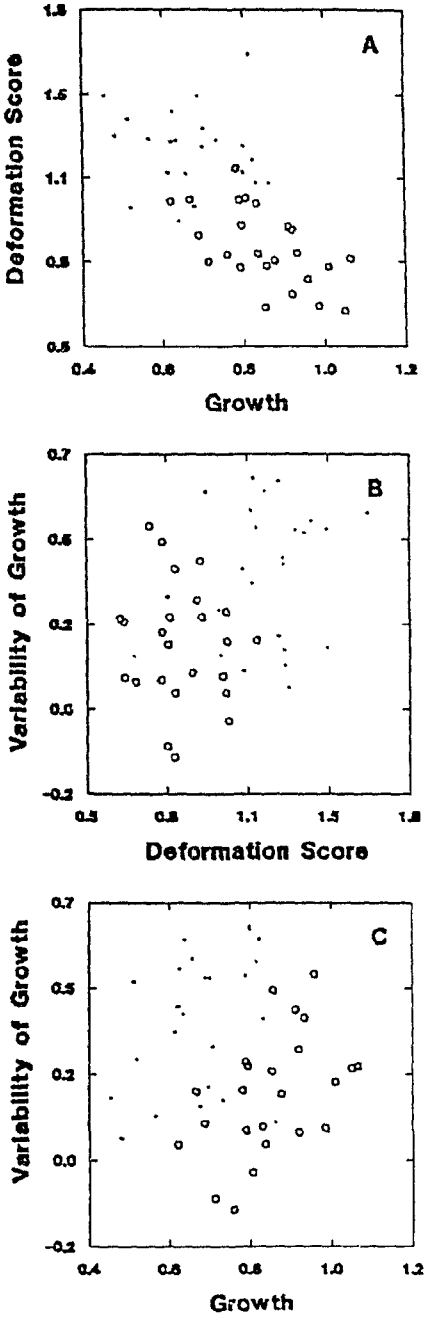


Fig. 4.5. Relationships among: A) deformation score (log number of deformed circuli) and growth (% log mm/day) B) individual variability of growth (log of variance of circulus spacing) and deformation score C) individual variability of growth and growth. Filled circles= regime FE, open circles= regime CE (n= average 16 per regime family combination).

5 Discussion

This chapter presents a practical method of estimating developmental and growth stabilities by measuring scale morphological features. The results demonstrate how indices of developmental and growth stabilities can be used to characterize fish merit. A composite index combines 3 criteria of relative merit, the example provisionally defined herein is fish which grow fast and have high developmental and growth stabilities. A composite definition of merit extends definitions that are based on growth alone. It is also, in a bioeconomic sense, more realistic and consistent with what is considered to be superior merit in other crops and domesticated animals.

Growth, developmental stability and growth stability may be genetically and environmentally correlated, or they may not. Each has a genetic variance and a heritability and they can be considered separate traits, both from a genetic and an economic viewpoint. As indices of economic merit, both developmental and growth stabilities have value as they may ensure viability of broodstock over generations, and improve predictability and reduction of risk. However, the actual bioeconomic strategy will depend on the degree of environmental variability in the rearing system (Jinks and Pooni 1988) and on the extent of genetic correlations among variables. The underlying genetic correlations are important

because 1) negative genetic correlations between variables can constrain selection, or 2) positive genetic correlations between variables can result in inadvertent selection of an undesirable trait (Rosielle and Hamblin 1981; Lande 1979; Jinks and Pooni 1988).

Developmental stability, as measured by the inverse of deformation scores, reflects a relative physiological ability to adhere to a genetic plan. Animals which have high levels of developmental stability across a range of environments are presumably desirable breeders, other traits being equal. With respect to growth and individual variability of growth, it is not immediately clear whether growth stability across a range of environments is economically or biologically advantageous, when measured at the family level. From an applied or practical standpoint, the possible strategies must also include socioeconomic goals. For example, growth stability is advantageous if it is desired that families are insensitive to the environment. Growth instability is desired if the goal is to enhance the economic security of small scale fish farmers by selecting for local strains that do best in local environments or respond well to added food (Roger Doyle, unpubl. data). Stability is desired if environmental variation is considered to be a stress, such as poor water quality, but may not be desired if environmental variations are considered to be an advantage, such as added food. In

agriculture, signals are mixed because better strains are often referred to as "unstable" in stressful environments yet "more responsive" to added inputs (Jinks and Pooni 1988).

From an evolutionary perspective, either negative or positive genetic correlations between growth and individual variability of growth may be adaptive; in fact the dichotomy defines the 2 possible strategies an organism can adopt in the face of environmental change (reviews in Bradshaw 1965; Schlichting 1986). Via (1987) maintains that genetic variation in response to the environment allows the evolution of phenotypic plasticity (one genotype resulting in adapted phenotypes in response to environment) which is itself an adaptation. If the genetic correlation between growth rate and growth stability is negative, then the genetic correlation may reflect the possibly adaptive ability to stabilize growth in a heterogenous environment. On the other hand, if growth and growth stability are positively correlated (e.g. in Pinus attenuata; Strauss 1987), then sensitivity to the environment reflects the possibly adaptive ability to opportunize by increasing growth in favorable environments (Strauss 1987). If fish breeders select for phenotypic stability across environments when the correlation between growth and variability of

growth is positive, there is a risk of losing a valuable adaptation,that is the ability to respond optimally and maximize growth when conditions permit (Via 1987).

The results show that growth is negatively correlated with growth stability at the individual level (among social micro-environments), yet positively correlated at the family level (among environmental macro-environments). In addition, the family correlation between growth and individual variability of growth is nonsignificant in the restrictive regime FE yet significantly positive in the nonrestrictive regime CE. I favour the explanation that, at the individual level, each fish is reacting to other fish. For example, the growth of small submissive fish may be sensitive to that of large fish; access to food will be more variable for small fish and consequently their growth will be more variable. However, in each family, the dominant fish are capable of enhancing growth rate when conditions permit. The ability to do so differs among families, that is some family individuals can respond to good environments better than others. The results suggest an opportunistic response at the family level to the nutritional environment. Hypothetically, within each family, fast growers will maximize growth, until they are limited by their physiology or the environment, compensate by slowing growth, and the result is a more variable growth pattern.

A positive correlation between growth and individual variability of growth at the family level is of importance to genetic improvement programs because families or strains are used to compare across environments. That is, individuals are not replicated across environment. Therefore, the family or strain correlations should be considered when developing a bioeconomic plan. In any event, fish breeders should choose fish based on the underlying genetic correlations among growth and developmental and growth stabilities that are established in the future rearing environment.

The response of variables to genetic and environmental factors is expected to change under different types and severity of stress. The purpose of this chapter is to demonstrate how 3 criteria can be combined to reflect overall merit of growth at individual and family levels. Genetic improvement programs, involving conservation of natural populations, maintenance of genetic diversity, and genetic enhancement of desirable traits, are of critical importance to the Nile tilapia industry (Pullin and Capili 1988). However, genetic selection is presently limited by the lack of accurate research methodologies designed to statistically minimize environmental sources of variation (Tave 1988).

I present a method to evaluate tilapia merit. Composite indices of merit have 3 potential applications: 1) as an indicator of environmental stress, the index could be used to detect sub-lethal stress of broodstock over time or between aquacultural systems. The source of stress can be inferred by coupling the results with technical information about a particular system (e.g. poorer quality of food, change in pH, introduction of competitor species); 2) as an indicator of genetic stress, e.g. inbreeding to compare populations in a single environment; 3) as a selection index to compare individuals within environment X population combinations.

An immediate application is to compare levels of abnormal scale morphology among aquaculture systems in order to diagnose stressful environments. The eventual use of the scale morphological technique is in a quantitative genetic experiment designed to identify genotypes on artisanal farm systems. Whatever selection procedure is used, it will be less costly in the long run, to enhance and exploit the natural biology of tilapia by selecting for a composite merit of growth and stress resistance. A composite index incorporates 3 important economic traits, and may differentiate individual, family and population overall merit more accurately than any one trait alone.

Chapter 5. General Discussion

1 Regime

The expected effect of dietary regime was that both growth and developmental stability would be higher in regime CE. FA and deformation scores were used as indices of developmental stability. The only unexpected result was that developmental stability, as measured by FA was lower in regime CE. However, I believe that the circulus deformation scores are a better index of developmental stability.

Both indices are the result of at least partly different developmental processes. Relevant to this thesis is the amount of developmental error detected by each method. The amount of circulus deformation index might be a more sensitive index of developmental error. Each circulus ridge deposition is an isolated developmental event that takes only a day or two, and I count each one as deformed or not deformed. The developmental process of the FA morphological characters is either bone elongation or an increase in tissue volume/surface area which register changes more slowly than circulus deposition. All developmental processes are reduced during slow growth but since FA is the difference between left and right sides, the FA measure is also reduced, whereas a circulus deposition is still recorded. At the limit, a stress that allowed no growth at

all would obviously not generate any FA or circulus deformations. Given the difference in methods, the signal of developmental errors is much weaker for FA than for circulus deformation. The different resolving power of developmental events accounts for the different results between FA and circulus deformation; the latter is simply a much stronger signal.

As the results pertain to field techniques, the relationship between FA and dietary regime is not resolved by these data. FA could be investigated in less severe stresses. However, given that fish did grow in regime FE and that the FA variation was artificially high in my experiment as all other forms of environmental variation were reduced, FA is probably not a good indicator of dietary stress in artisanal systems. Deformation scores, however, have great potential and should be investigated as an index of other forms of stress.

2 Inbreeding

Inbreeding depression of production traits has been documented in fish (in rainbow trout (Salmo gairdneri, Aulstad and Kittelsen 1971; Kincaid 1976a,b,1983; Gjerde et al 1983; in common carp (Carpio cyprinius) , Moav and Wohlfarth 1968). Inbreeding depression is caused by a loss

of genetic variation and has been shown to cause a decrease in fitness characters (Wright 1977; Falconer 1989). The actual mechanism is hypothesized to be due to an increase in expression of deleterious alleles or by increased homozygosity at overdominant loci resulting in a loss of heterotic effect (reviewed in Mitton and Grant 1984). The latter explanation is corroborated by the fact that heterozygosity is often positively associated with fitness (reviews in Zouros and Foltz 1987 and Koehn 1989). My results show that Nifi and Israel strain were both affected by one generation of brother-sister mating in some variables but only to a small degree. Growth was depressed in inbred Israel yet outbred Nifi grew slower than inbred Nifi. In fact outbred Nifi grew more slowly than all other lines. Developmental stability, as measured by both body and scale morphologies, was minimally, but significantly, higher in outbred lines than in inbred lines.

How important is inbreeding to Tilapia aquaculture? A loss of genetic variation can be caused by "bottlenecks" (when the effective population number is severely reduced) or by mating between close relatives. However, there are many cases in which there are no deleterious effects of inbreeding (Brewer et al 1990). There are reports of a loss of genetic variance in hatchery salmonid stocks (e.g. Cross and King 1983) however only minimal effects of inbreeding were observed in rainbow trout (Salmo gairdneri) (Gjerde et

al 1983) and in channel catfish (Ictalurus punctatus) (Bondari and Dunham 1987). The benign effects of inbreeding are ascribed to 1) a release of additive genetic variance, due to a breakdown in epistatic effects 2) a decreased genetic load in a bottlenecked population, as a result of the initial low frequency of deleterious recessives which are subsequently lost through random genetic drift and 3) the bottleneck caused adaptation to inbreeding. These are explained below.

1) Purely additive genetic variance is theorized to decrease within lines due to random genetic drift. Dominance variance, however, is dependent on initial gene frequencies and if the recessive allele is rare, the within line additive variance actually increases during initial inbreeding, peaks at F=0.4-0.5, and decreases thereafter (Robertson 1952). However, single bottlenecks may not cause such detrimental decreases in genetic variability as previously theorized. Firstly, Lande (1988) notes that only $1/2N_e$ of heterozygosity of additive genetic variance is lost per generation due to random genetic drift (where N_e is effective population size). Secondly, Carson (1990) argues that bottlenecks may actually cause the release of additive variance within lines, previously limited due to the epistatic interaction of coadapted gene complexes. If a population undergoes a severe bottleneck, the gene complexes are disrupted, recombination occurs and genes act additively

once again and are available for natural selection. The beneficial effects of such an event assume that mostly highly heterozygous and fit individuals survive a bottleneck; that is, the average allele survives, not the rare deleterious ones (Carsons 1990; Parsons 1990a). These capable founders form a new population and if left relatively undisturbed, will regain former genetic variability through recombination, mutation (Lande 1988) and the occasional migrant (Falconer 1989). Bottlenecks can be seen as a weeding out process but the added benefit of increased additive variance, through loss of dominance or breakdown of epistatic complexes, mitigates inbreeding effects on small populations. So, although allozymic variability can decrease, additive genetic variance can be released. The amount is dependent on the recombination rate and size of founder population. Lopez-Fanjul and Villaverde (1989) observed increased additive variance within inbred line and a significant response to selection for egg-pupa viability. There was no significant response to selection in the non-inbred control lines.

2) Brewer et al (1990) explored similar ideas about the frequency of deleterious alleles in small populations; their argument of deleterious allelic effects differs from Carson's, but the end result is similar ; bottleneck populations do not always show decreased fitness. Brewer et al (1990) argue that even in the absence of natural

selection on deleterious alleles, the population size experienced in bottlenecks results in a decreased genetic load because the frequency of deleterious alleles is so low in small populations. These rare alleles are lost more readily in small populations through a result of random genetic drift.

Brewer et al (1990) examined several populations of Peromyscus with varying bottleneck histories. Mice from insular populations and from panmictic populations were purposely inbred; the prediction was that inbred lines from insular populations would show adaptation to inbreeding. Although they did observe inbreeding depression in various traits, their results do not accord with the prediction that insular populations should show adaptation to inbreeding relative to larger panmictic populations. Some populations suffered 100% juvenile mortality after F=0.45 while others showed no reduction in litter size at F=0.39. In the end, inbreeding depression was observed to be random among populations and could not be ascribed to population of origin (insular or panmictic). The authors suggest that inbreeding effects are caused by relatively few genes. Founder populations retain these genes at random, therefore the effects of bottleneck on inbreeding depression are random among founder populations.

3) Angus and Shultz (1983) observed no difference in asymmetry levels among heterozygotes and inbred in Poeciliopsis. Inbreeding commonly occurs in natural populations of Poeciliopsis and the authors hypothesize that poeciliid evolution allowed for inbreeding while simultaneously selecting for developmental stability. In other words, the relationship between FA and heterozygosity may depend on the species evolutionary history. In outbreeding panmictic populations, rapid inbreeding would be detrimental. In more isolated systems, the genome may evolve to accommodate inbreeding and retain developmental stability. That is, rapid inbreeding or homozygosity, may not seriously disrupt developmental stability because evolution has removed the homozygotes of lowered fitness.

If inbreeding depression is dependent on the genetic histories of a population, then inferences of empirical studies can only be applied to the sample populations. Barker (1988) has succinctly stated the efforts of empirical works

"...a single natural population is a single replicate of the effects of natural selection and genetic drift...".

Both Bondari and Dunham (1987) and Gjerde (1988) remark on the random nature of inbreeding effects among families; they attribute the lack of statistically significant effects of inbreeding to low family sample size. Still, the loss of genetic variation is a potential problem in genetic

improvement programs. Inbreeding theory is backed by numerous examples of a positive association between heterozygosity and fitness traits, developmental stability included. A decrease in genetic variation in isolated populations can lead to a decrease in a population's ability to respond to environmental stress (Lerner 1954; Levin 1970; Leary et al 1985). The random nature of inbreeding effects among families and lines is theoretically and empirically established, the only recourse for fish breeders is to safeguard against it.

Tilapia (Oreochromus niloticus) are naturally distributed in the Nile and east, central and west Africa; man has introduced Tilapia to warm climates on all continents (Philippart and Ruwet 1982). The Nifi population at Binangonan originate from Egypt, tilapia were taken to Japan in 1962. The Prince of Japan donated 50 fingerlings to Thailand in 1965 of which only 19 pairs survived to reproduce (S. Uraiwan, pers. commun.). In 1987, 50 pairs were given to Zubaida Basiao of Binangonan, Philippines, 500 fry were produced from mass spawning and formed the first generation. I used the first generation as parents and the second generation in the thesis experiment. From this scant history, it appears the Nifi strain has gone through at least one bottleneck in 1987. The origin of the Israel strain is less certain. The strain was introduced to the Philippines from Israel via Thailand in 1972 and donated to

Binangonan in 1986. It is suspected that there has been introgression of Tilapia mossambicus genes as the result of a big flood in Nueva Ecija in the Philippines. From these scant histories, only one thing is certain, the Nifi strain has less genetic variation, possibly as a result of the bottleneck in 1987. My results indicate that the Nifi strain did not grow as fast and suffers more from inbreeding (with respect to developmental stability) than the Israel strain.

3 Heterosis

Strain hybridization has potential for aquaculture to improve poor quality strains or capitalize on the effects of heterosis. For example, Ferguson and Drahushchak (1990) found a positive association between disease resistance and heterozygosity. Heterozygous rainbow trout better survived a bacterial gill disease and they were larger than their less heterozygous counterparts (Ferguson and Drahushchak 1990). Ayles and Baker (1983) observed significant genotype*environment interaction in rainbow trout populations in different lakes. The authors note that superior hybrid strains could be created for aquaculuture by combining the best abilities of separate stocks. However,

Gjerde (1988) has suggested that hybridization between inbred lines of salmonid species is too costly due to the poor viability of highly inbred lines.

Heterosis, when hybrid progeny perform better than the mid-parental mean, is caused mostly by dominance effects in crop plants (as opposed to epistasic (interacting gene loci) effects) (Geiger 1988). The expectation is that increased heterozygosity of hybrids should increase growth and developmental stability as heterozygosity is proportional to fitness traits. Both hybrids in my experiment grew faster than the mid-parental mean but neither grew faster than the Israel strain. Hybrids had higher developmental stability than the midparental value, as measured by deformation scores, but only marginally by level of asymmetry. My results show that the Nifi strain might well be improved by gene introgression of the Israel strain.

4 Genetic Improvement strategies

Tilapia are hardy fish yet several researchers contend that the quality of broodstock in the Philippines has deteriorated (Bimbao and Smith 1988; Tayamen 1988). The causes are suspected to be (1) the practice of marketing the large fish has left smaller more inferior fish as breeders (R. Eguia pers. commun.) and (2) introgression of unwanted

mossambicus genes (Macaranas et al 1986). Efforts are now being made by government and nongovernmental agencies to expand genetic improvement programs (Abella et al 1986; Pullin and Capili 1988; Tayamen 1988; Basaio and Doyle 1990). A central dilemma in the South East Asian aquaculture community is whether to cultivate one strain that is insensitive and grows reasonably well in all environments or local strains that are sensitive and maximize growth in specific environments. Whether sensitivity to the environment is desirable depends on the trait, and the environmental variable. For example, growth that is insensitive to stochastic weather yet sensitive to nutritional input or improved water quality is desirable (Jinks and Pooni 1988).

Fish grow in a range of environments which is important to fish breeders for 2 reasons: fish breeders need to know if (1) genotype ranking and response are similar across a range of environments and (2) how the correlation between traits (e.g growth and fecundity or growth and stress resistance) changes across environments. It is often observed that genotypic differences (Kohane and Parsons 1988; Koehn 1989)and that the correlation between fitness traits (Govindaraju and Dancik 1987; Gebhardt and Stearns 1988) are more easily detected under stressful conditions.

(1) Genotype* environment interaction: In chapter 4, I argued that genetic variation of response to different environments is important as it could allow the evolution of adaptive response to local environments (Via 1987). If artificial selection programs select for a lack of response to environmental variation, then genotypes lose the ability to respond to novel environments. The population is thus genetically constrained (Levin 1970). Indeed, some researchers contend that it is the function of an organism's response across environments which changes through evolutionary time, and not the trait itself (e.g. Dobzhansky 1951 c.f. Caswell 1983). Doyle et al (submitted) argue that selecting for local strains would actually increase genetic variation between localities and contribute to genetic conservation in a dynamic manner. A high between site genetic variance would offset the loss of genetic variance often associated with the one strain/ monoculture selection strategy.

Nonetheless, it is important to consider the underlying correlation between growth and growth sensitivity (response to environment) because it will change under selection. The direction of the change is determined by the initial genetic correlation and by the quality of the environment in which selection takes place (Charlesworth 1984; Jinks and Pooni 1988). If the genetic correlation between growth and sensitivity is positive, it means that

the fish have an adaptive responsiveness to the environment; if negative, responsiveness is maladaptive. In agro-industries, selection for fast growth in good quality environments has resulted in maladaptive sensitivity to the environment (Plucknett and Smith 1982, 1986; Jinks and Pooni 1988). If the underlying genetic correlation between growth and growth sensitivity is maladaptively negative, an alternative selection strategy might be to eliminate the extremely sensitive but fast growing fish in order to retain the population's ability to respond to the environment. This would set up a situation whereby evolution of optimal response could occur. Before any selection program is undertaken, the genetic correlations between growth and growth sensitivity should be determined in good and bad environments (Jinks and Pooni 1988).

2) Correlation between traits: High stress resistance across all environments is desirable and is linked to growth sensitivity because selection for high growth and stress resistance could increase the adaptability of a fish strain. That is, selection of fast-growing, stress resistant fish would promote the evolution of a positive correlation between growth and growth sensitivity. But what is stress resistance? There is more than 1 mechanism responsible for stress resistance. Stress in one environment is not stress in another. Even within one environment, stress resistance takes many forms. Individual immune response to the same

viral or bacterial infection can occur in many ways: mucous secretion from the skin, antibody formation, increased production of lymphocytes or white blood cells (Chevassus and Dorson 1990). The key to identifying a trait which embodies stress resistance is of utmost important to fish breeders. Physiological indicators, such as adrenal hormone levels, would be suitable but are difficult to measure in the field. Hoffmann and Parsons (1989) have hypothesized that increased stress resistance is associated with lower metabolic rate. They provide evidence of an association with lower basal metabolic rate an various fitness traits in Drosophila.

Metabolic rate may be the key to the mechanism of stress resistance. In the interim, before physiological indicators evolve to a practicable field technique, gross morphological features, such as body and scale morphologies can serve to identify stress resistant fish.

The choice of selection method depends on finding out the relative performance of strains across a series of environments and on genetic correlation among fitness traits. If one strain does consistently better in all environments (without costly technical inputs), then clearly, that strain should be cultivated. If strains perform differently in different environments, then it might be better to develop locally adapted strains. The advantage of cultivating one stable strain is that there would be a

larger gene pool, thus the danger of inbreeding is reduced. The disadvantage of cultivating one strain is that the natural farm ecosystems of Tilapia are extremely variable. If selection has to be done under controlled laboratory systems, the fish won't nessecarily perform well under farm conditions. This would lead to the danger of augmenting fish performance by exporting laboratory conditions to the field. Laboratory conditions are typically highly controlled and artificial, in order to decrease the environmental component of variation relative to the genetic component of variation. Sustaining the growth of laboratory selected fish in field conditions could eventually require the use of antibiotics, supplemental food, chemical fertilizer and pesticide inputs, all of which are too costly for the small-scale fish farmer. The economics of supporting a natural resource through technology render the potentially self-reliant fish farmer dependent on costly technical inputs. The advantage of cultivating many local strains is that each strain would evolve optimally in site-specific conditions and genetic variance between systems is high. The disadvantage include the danger of inbreeding and creating limited gene pools. However, site-specific conditions are particularly important given the vast array of tropical ecosystems, stagnant ponds, rice paddies, eutrophic lakes and the fact that fish respond so readily to environmental conditions.

My results show firstly, that genotype*environment interactions were not important in the analysis of growth but were, in the analysis of developmental stability. Secondly, they show that the correlation between growth and developmental stability was different in each regime. The results demonstrate the value of an additional variable, developmental stability, in evaluating merit. Genetic improvement programs could easily embark on selection for growth alone while neglecting the importance of developmental stability, thus losing the opportunity to create a truly superior strain. Whether the results can be applied to other systems or not, they advocate the testing of genotypic merit in a range of environments, or at least in the environment in which a strain will eventually be cultivated.

In a final review, the importance of genotype*environment interaction in tilapia has not been fully documented. Based on the experience of monoculture agriculture, the fact that genotype ranking and genetic correlations among traits can vary according to environment, and that it is of higher social benefit to develop local strains, a cautious approach would be to test both growth and stress resistance in a range of environments before deciding whether to breed one or many strains.

Appendix 1: Feed Ingredients of Dietary Regimes

Nutrient Composition of COMMERCIAL FOOD PELLETS*
(Manufactured by Universal Robina Corporation, Pasig, Manila Philippines)

Ingredient	Percent
Crude Protein	26 %
Crude Fiber	9
Crude Fat	4
Moisture	13
Ash	12

Nutrient Composition of RICE BRAN
(Purchased at Binangonan Market, Phillipines)

Ingredient	Percent
Crude Protein	6.61 %
Crude Fiber	21.83
Crude Fat	3.64
Ash	15.52
Dry Matter	14
Nitrogen Free Extract	39.79

*Information from Cory B. Santiago, fish nutrition scientist at Binangonan, SEAFDEC, Rizal, Philippines.

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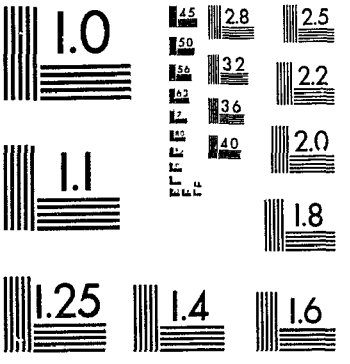

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

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