Study of Population Genetics As a Tool for Determining Larval Dispersal and Recruitment of Marine Species

Laporan Penelitian

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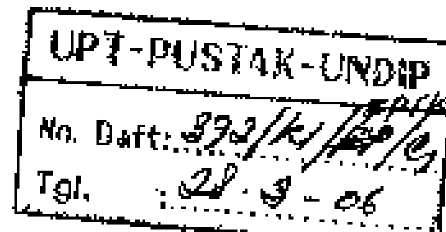
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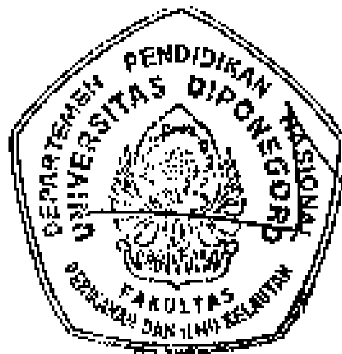

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Abstract

The population genetic involve the study of individual characteristics and cannot be done by grouping. The electrophoresis method is one of the most popular method in determining individual characteristic.

Through study of population genetics, the genetic differences between species from two different locations, can be differentiated. However, the gene flow or the larval dispersal cannot be detected by using this method. This limitation is due to the fact that there are many factors regulating the gene flow, and these factors consistently change from time to time. These factors are the ocean circulation, the natural phenomena, the geographic isolation, the natural selection, and the geologic evolution.

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1.0 Introduction

The life history of every species in this world differs to one another. This fact works also for marine species. This life history includes the age and growth, the spawning season, the requirement of food, temperature, salinity etc. There is, however, one important factor in every marine species life history which will eventually affect the evolution of the species, and this factor is the larval stage.

Holm and Bourget (1994) explained that the population genetics structure in sessile marine invertebrate would depend on the life history of the species and the spatial scale of observation. There are two types of larval stage namely the planktonic larvae, and the crawling juvenile. The planktonic larvae allow high level of dispersal, which result in low level of genetic variation among local population, but high genotypic diversity within population. On the contrary, the crawling juveniles are highly restricted in their dispersal and form a set of closed and inbred local population. This result in high level of genetic variation among local population, but less genotypic variability within population (Hoskins, 1997).

The geographic features are one of the most important factors that influence the larval dispersal of a marine species. Another factor that determines the flow of the planktonic larvae is the ocean current. These two factors will actually determine the direction of rec uit settlement, and will lead to the determination of population and evolution in a certain area.

The geographic isolation is very important in affecting the differentiation of the local gene pools. The greater the distance between them, the less gene flow will occur (Cousin, 1996). On the other hand, the ocean current is also important , because it determine the biological productivity pattern. Nevertheless, it is difficult to determine such pattern in the ocean, because the dispersal of planktonic larvae is a function of passive transport and

active swimming by the plankton (Bucklin, 1995). Furthermore, Bucklin explains that it is even more difficult to do a direct observation of this larval dispersal. This dilemma arise due to the condition that the planktonic larvae are small in size, numerically abundant, and the wide distance of their drift.

In order to measure the dispersal of planktonic larvae, many scientist have chosen the approach to infer dispersal from the population genetics of a species (Bucklin, 1995). Bucklin (as quoted in her essay), further explain that the reason to choose this approach is because "dispersal is the exchange of individuals among conspecific populations, which acts to genetically homogenize the population (assuming the transported individuals eventually reproduce in the destination population, which is the proper definition of dispersal)". Bucklin also mentioned that the gene flow would act in decreasing the population genetic differentiation (equal opportunity in mating one another).

The genotypic structure of a population can be determine by either the frequency of genes and forces that affect their frequencies (such as migration, mutation, selection, and genetic drift), or by the system of how an individual finds a mate (Gall, 1987). Moreover, Gall revealed that the mating system can be random, which means that every individual have an equal chance to mate with any other individual in a population. Gall in addition to above fact, suggested that mates can also be chosen according to either their genealogical relationship or their phenotypic resemblance.

Johnson and Black (1982) explained in their essay that with planktonic dispersal, the recruits of a certain area might not be the relatives of the local individuals. Therefore, this situation can result in a decreased differentiation among sites (Scheltema, 1971; Berger, 1973; cited in Johnson and Black, 1982).

In this paper the basic description of population genetics and the method of electrophoresis procedure will be explained. This paper will also discuss about the previous research from several scientists whom have studied the larval dispersal of marine species by using population genetics as the determination tool.

1.1 Population genetics

Population genetics is a study of individual characteristics which cannot be done by groupings (Bucklin, 1995). It may not look possible to trace an individual plankton's dispersing destination, but it is possible to analyze the proportion of larval immigrant individually in a certain area on an oceanographically relevant time (Bucklin, 1995).

In order to examine whether or not a particular marine species are related to one another, although they differ in location, the population genetic study is put to use. This explains why the geographical structure and the ocean current are important factors that influence the geneflow of the planktonic larvae. The stronger the current generally results in a higher gene flow, while a weak current will commonly result in a lower gene flow.
A small, isolated geographical area will generally have a low gene flow, whereas a wide and open area will usually have a higher planktonic gene flow. Organism with little or no gene flow between species living in different area, have the tendency to go on their own evolutionary way, and adapting with their own environment (Cousin, 1996).

In measuring the gene flow of an individual, it is important to understand the basic principal of molecular analysis of gene flow pattern, which involves the fundamental chemical substance of a gene namely deoxyribosenucleic acid or DNA. According to Utter *et al* (1987), "DNA is a giant molecule constructed in a so called double helix spiral ladder".

The ladder are deoxyribose (altering sugar) and phospate, and the rungs are pairs of basis: adenin (A), guanine (G), thymine (T), and cytosine (C), which paired on either AT (or TA) or CG (or GC) (Utter *et al*, 1987)

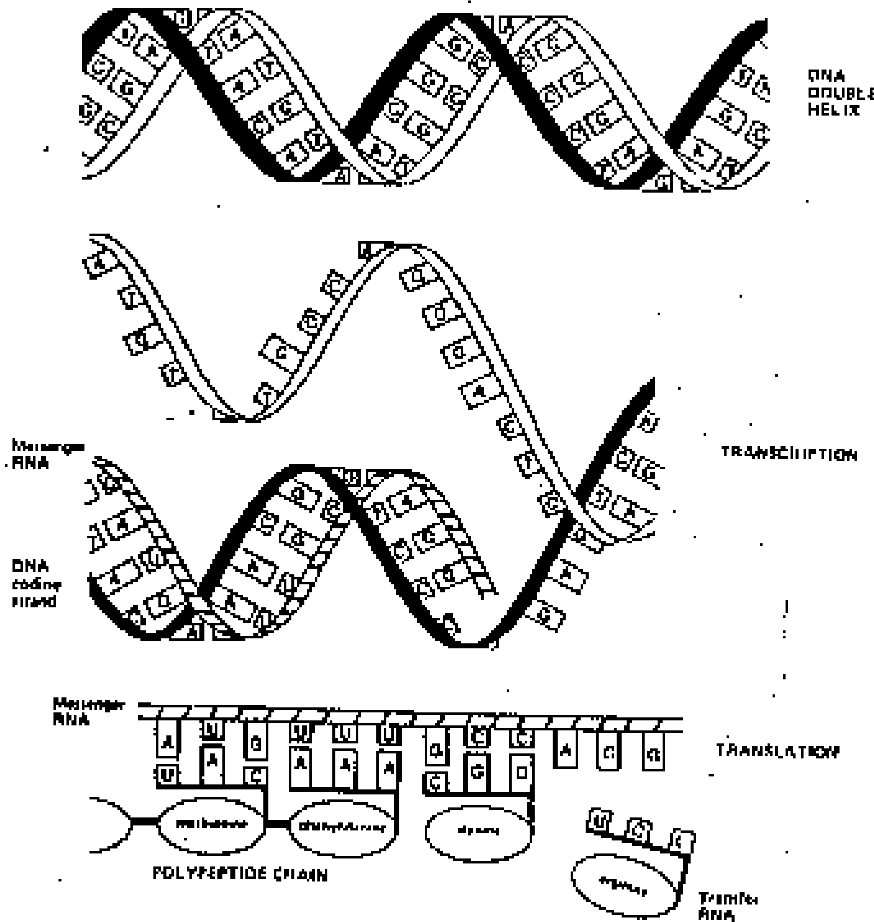

Fig 1. Molecular processes relating base sequences of DNA to amino acid sequences of polypeptide chains (protein). Messenger RNA, synthesized during transcription, provides a template for the synthesis of polypeptide chain during translation. The bases in DNA are cytosine (C), guanine (G), adenine (A), and thymine (T). In RNA, the base Uracil (U) replaces thymine (T) of DNA (Utter *et al* 1987)

Utter *et al* further describe the two steps in uncoding of the segment of triplets in DNA molecule into amino acid in protein:

a) Transcription

This process involves the transcription of genetic information from DNA into RNA (ribonucleic acid), commonly called as mRNA (messenger RNA). It is called messenger RNA simply because it carries the coded information of DNA, from the nucleus to the cytoplasm, where the protein production takes place. Messenger RNA, unlike DNA, is single stranded and very small.

b) Translation

Since the mRNA is so small, it is able to pass from the nucleus to the cytoplasm through small pores in the nuclear membrane. This process involves the pairing of triplets on mRNA with triplets from transfer RNA (tRNA).

The base sequence of DNA and protein structure are similar in the way that they are both made of different components of amino acid (Utter *et al*, 1987) These amino acids are connected together and form a polypeptide chain (Fig. 1). Utter *et al* further explained that the base G, A, C, and T, can be arranged in 64 different ways (in combination of three) and form a particular amino acid. Somehow, a mispairing of amino acid can occur which will result in mutation, and when it is passed to the next generation, this will be the original genetic variation (Utter *et al*, 1987). Nevertheless, if there are no disturbances or mispairing, the genotype frequencies will remain constant from generation to generation (Richardson *et al*, 1986).

According to Davis (1996), the Hardy-Weinberg law states that " the allele frequencies will remain constant from generation to generation; genetic variation doesn't disappear with time " (Wallace, 1981; Lincoln *et al*, 198?; quoted in Davis, 1996). There are several assumptions to be met if a population is found to be in the Hardy-Weinberg equilibrium:

a) There are no immigration or emigration of genotypes
b) There are random mating between genotypes
c) There are no selection between genotypes
d) There are no mutation
e) There is a large population

(Richardson *et al*, 1986; Hartl and Clark, 1989; May and Kreuger, 1990; cited in Davis 1996)

1.2 Electrophoresis

According to Cousin (1996), electrophoresis can be defined as "the movement of charged particles in solution under the influence of an electric field". This electrophoresis procedure will permit a rapid and reliable identification of protein variation reflecting simple genetic differences (Utter *et al*, 1987).

In her essay, Cousin mentioned that the electrophoresis setup includes: a power supply, and the electrophoretic chamber (which the electricity will travel through, and holds the gel). The agarose gel (containing samples in wells to be separated). is submerged in buffer within the electrophoretic chamber. The protein will migrate as the cause of current from the power supply (smaller particles will migrate much faster than larger particles).

Unlike human, the material used for electrophoretic procedure in marine species, instead of using blood, the muscle tissue and liver are used. This is based by the fact that the fish erythrocytes are fragile and it is difficult to produce and preserve discriminating antiserum (Hodgins, 1972; cited in Utter *et al*, 1987).

The basic working system of electrophoresis was the effect of electric field on charged particles including protein (Purnet,1816; Quincke, 1861; Hardy, 1899; cited in Richarson *et al*, 1987). Richardson et al further explained that the amino acid might have acidic (COO^-), or basic (NH_4^+) groups. Therefore, the protein will carry either positive charge, negative charge, or no charge at all. For example lysine, arginine, and histidine are positive, whereas aspartic acid, and glutamic acid are negative (Utter *et al*, 1987).

The occurrence of allelic differences at a protein-coding locus (example:different forms of gene), will result in the changes of the protein charge. This can be identified through the gel electrophoresis. The most common gel used is strach gels, acrylamide gels, agar gels, and cellulose acetate gels (Richardson *et al*, 1986).

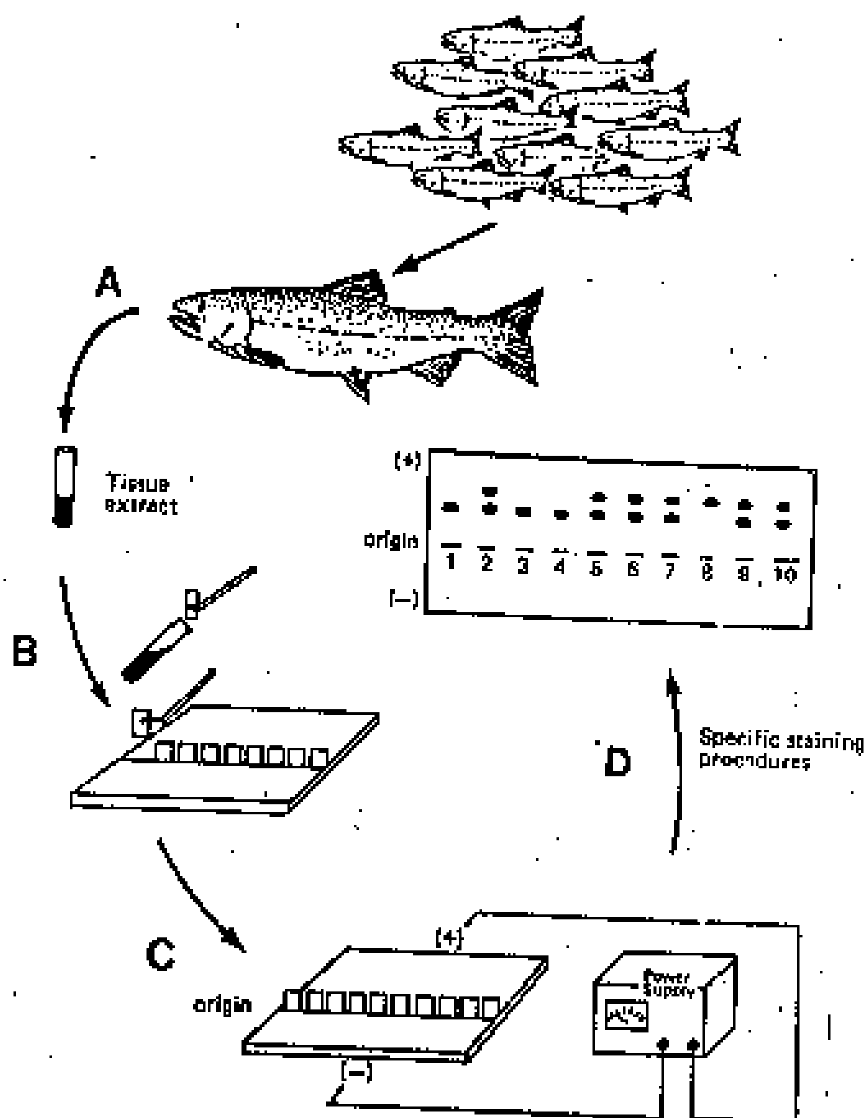

Fig 2. Standard steps for obtaining genotypic data from electrophoresis (modified from Gharet and Utter, 1982). (A) Crude protein is extracted from tissue such as muscle or liver. (B) Extract from each fish is introduced individually to gel by filter paper insert. (C) Different form of a particular protein often move different distances from the point of origin when electric current is applied because they have no identical electric charges. (D) These forms are readily identified by a specific stain for each protein type. Specificity in staining permits identification of both the activity and the exact location of a particular protein from an individual fish from a complex mixture of proteins in each protein extract - Intensities of banding patterns do not reflect differences of gene dosage in this depicition (Utter *et al*, 1987)

The following electrophoresis procedure is taken from Davis (1995) in her population genetics research:

1) Tissues (liver and muscle) are dissected and store in 1.5 ml Eppendorf tubes at -80°C until it is used.
2) Samples are homogenized and centrifuged at 4°C, 500 rpm, for 10 about 10 minutes (to pellet the cell debris while leaving the protein in solution).
3) The prepared starch gels is cut 5 cm from the cathôdal end of the gel.
4) The tissue supernatant are absorbed into a filter paper wick, and placed on to the cut end of gel by usi g forceps.
5) The paper wick which are blotted in Brophenol Blue are placed at far right, centre and far left of gel (this is to determine how far the samples had moved through the gel).
6) After samples are loaded, the separated gels are pushed back together and the gels are blotted to remove excess samples.
7) The gels are then covered with plastic food wrap
8) The gel is placed in an electrophoresis chamber containing appropriate electrode buffer.
9) Direct current was runned using Pharmacia Electrophoresis power supply model ES 500/400
10) Gels were run until Bromphenol Blue tracking dye had migrated 5-7 cm from the origin.
11) Gels were then sliced horizontally, removed using plastic sheet and placed into plastic tray with lid.
12) The sliced gel are stained and screened.

Different species have different sequence of amino acid, therefore the type of stain being used are also different from one species to another. Many scientists have studied population genetics for decades, consequently information regarding which kind of stain to be used can be found in other literature.

1.3. Statistical Analysis

In order to measure the gene flow of a population in a certain area, first thing to measure is the determination of the frequencies of the variants of genes in each population by a statistical examination (Bucklin, 1995).

1.3.1 The F - statistic (F_{ST})

The F-statistic (F_{ST}) is a statistical approach to describe the distribution of genetic variation between population and within population, and the values varies between zero to 1.0 (Bucklin, 1995). Bucklin further include the quotation for F_{ST} follows:

$$F_{ST} = \frac{\sum p\left[\frac{V_p}{p(1-p)}\right]}{n}$$

where,

Vp: variance of frequency in allele p

p : mean allele in all samples

n : number of alleles

1.3.2 Number of Individual Exchanged (Nm)

By using the calculated F_{ST}, the number of individual exchanged between two populations can be estimated as follows:

$$Nm = \frac{1}{F_{ST} \times 4}$$

where N: number of individual in a given population

m : proportion of individual and immigrants

(Bucklin, 1995)

Furthermore, Bucklin explained that in a homogeneous population, where the gene flow is high is indicated by the value of $Nm > 1$ (corresponding to the value of F_{ST} as < 0.25).