MARINE MOLLUSCS: APPLICATION OF MOLECULAR MARKERS FOR ESTIMATION OF EFFECTIVE POPULATION SIZE AND GENE FLOW

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ABSTRACT

The aim of this review is to present methods for estimation of effective population size and gene flow among populations of marine molluscs. The basis for the estimation is the existence of an adequate genetic marker, e.g. protein and/or nucleic acid marker. The method for estimation of effective population size is based on temporal variation of allelic frequencies or linkage disequilibrium, whereas the methods for estimation of gene flow are based on spatial variation of allelic frequencies. Applications of the various methods for marine molluscs will be presented.

INTRODUCTION

When studying marine molluscs, two features are important for understanding the genetic dynamics of the populations. Firstly, what is the effective population size and how important is the effective population size for the sustainability of the population, and secondly, how big is the gene-flow among populations and how necessary is the gene-flow for the sustainability. By saying this, it is obviously, that natural populations are considered, but it has to be emphasised, that the effective population size is also very serious for optimalization of aquacultural production of various sea-food.

Measurements of population size or migration between populations can be done by doing *e.g.* mark-release experiments. These direct methods are well established in fishery biology and ecology as such, but may be difficult to use for many organisms. A way to cope with these difficulties may be the application of indirect methods, which are based on genetic markers. The use of Mendelian inherited markers allows estimation of effective population size and gene flow between populations.

Effective population size is the number of individuals contributing to the next generation. For limiting loss of genetic variation, a population has to be of a certain size. A number of 500 breeding individuals have been suggested (Ryman & Utter 1987). However, this figure has to be considered with some precautions, because many natural populations have marked smaller size. When working with aquacultural populations, a low number in the brood stock is often the case. Breeding programs utilising the breeding individuals in an optimal way may overcome the small population size (Falconer 1989). A similar approach may be useful for conservation purposes. Again the population size very often is very low, and optimal mating set-up for the individuals may prevent loss of genetic variation by random drift. However, not all organisms are adequate for controlled matings and a method for increasing the genetic variation in the breeding population may be to introduce individuals from other populations to the breeding stock. If the newcomers are successfully mated with the original broodstock, the event is equal to a gene flow. This method may take place under natural conditions and may be important for the sustainability of the natural populations.

Gene flow is the transmission of genes from one population to another. Rather few individuals from one population are needed

to have an impact another population. However, the number of migrants and their breeding success in the receiving population and the size of this population are important for the resulting genetic variation. For planktonic species, which are releasing their gametes in the water column, gene flow is of minor interest, but for organisms with internal fertilisation gene flow may have an important influence. Organisms, living in isolated areas, may also be very dependent on gene flow for avoiding the effect of random drift and thereby loss of genetic variation in the population. Slatkin (1985a) presented an excellent review on the importance of gene flow in natural populations.

Both effective population size and gene flow have a demand for recognition of genes or their products. An important assumption for both concepts is that the genes used for the estimation are neutral, *i.e.* no selection does occur. Molecular markers are mainly considered to be neutral, and encompass nucleic acids and proteins. Especially, proteins (*e.g.* isozymes) have been used for estimation of population size and gene flow as illustrated by the examples presented in the paragraph on application.

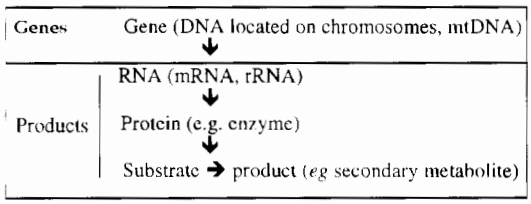
The aim of this review is to briefly describe the markers and the theory behind the methods, which can be used for estimation of effective population size and gene flow. The review will also provide examples on the application of the methods with the emphasis on marine molluscs.

METHODS

Molecular markers

Molecular markers of various sorts can be used for the estimation of effective population size and gene flow (*e.g.* Kjellsson *et al.* 1997). Most commonly used are allozymes (*e.g.* Neigel 1997; Simonsen & Kittiwattanawong 1998), but with the rapid developing methods for analysing nucleic acids (DNA and RNA) a new versatile tool is available (*e.g.* Neigel 1997; Parker *et al.* 1998). Chromosomes can also be used as markers, considering these as huge composite molecules. The relationship among the applicable molecular markers is depicted in Fig. 1.

Figure 1. Overview on molecular markers, genes and their products (RNA, proteins and chemical compounds present due to the metabolism). Abbreviations used are mtDNA = mitochondrial DNA, mRNA = messenger RNA and rRNA = ribosomal RNA



Effective population size N_e

Effective population size N_e is defined as the individuals in the population, which are contributing to the next generation (*e.g.* Hartl 1980). N_e is defined as the harmonic mean over time

$$\frac{1}{N_e} = \frac{1}{t}\sum_{i=0}^{t-1}\frac{1}{N_i}$$

where N_i is the population size to generation t, t is the number of generations. N_e for a population consisting of males and females all contributing to the next generation is

$$N_e = \frac{4N_m N_f}{N_m + N_f}$$

where N_m is the number of males and N_f the number of females contributing to the next generation. From this equation, it is seen that an uneven distribution of males and females may have a severe effect on the N_e. The implication of a small N_e may as mentioned in the introduction result in a loss of genetic variation, because random drift may act on the population, cause changes in the allelic frequencies and perhaps lead to fixation of an allele. The change in allelic frequencies can be expressed as shown below when assuming, that the alleles are neutral

$$F_a = \frac{1}{K}\sum_{i=1}^{K}\frac{(x_{i1}-x_{i0})^2}{x_{i0}(1-x_{i0})}$$

where x_{i1} and x_{i0} are the frequencies of allele A_i at a locus a time t and 0, and K the number of alleles. This expression has to be corrected for the sample size S_t and S_0 at time t and 0, so the final equation is

$$N_e = \frac{t}{2\left(F_a - \frac{1}{2S_0} - \frac{1}{2S_t}\right)}$$

as described by Pollak (1983) and Waples (1989).

Another possibility is to utilise linkage disequilibrium as described by Hill (1981). This method has been simplified by Waples (1991) (cited by Bartley *et al.* 1992) for unlinked loci and the expression for N_e is

$$N_e = \frac{1}{3\left(r^2 - \frac{1}{S}\right)}$$

where r is the correlation among alleles and S the sample size. r can be estimated from information on Burrow's composite measure of disequilibrium (Bartley *et al.* 1992; Campton 1987).

Gene flow

The most commonly used method for estimation of gene-flow is the application of Wright's indices also called F-statistics (Wright 1943, 1951). The indices are derived from a model on a population, divided into subpopulations. They express the probability for an individual to have identical copies of a specific gene. The relationship among the indices is as follows

$$(1-F_{IT}) = (1-F_{IS})(1-F_{ST})$$

where F_{IT} is the probability to have two identical copies of a gene in an individual when considering the total population. F_{IS} is the probability to have two identical copies in an individual relative to its subpopulation. F_{ST} is the probability to have two identical copies due to genetic subdivision relative to the total population. F_{IT} and F_{IS} are also called fixation indices and may be negative, whereas F_{ST} is an expression for the subdivision of the population and may have values from 0 to 1. F_{IS} is estimated as where H_{obs} is the fraction of observed

$$F_{IS} = 1 - \frac{H_{obs}}{H_{exp}}$$

heterozygotes and H_{exp} is the fraction of heterozygotes expected according to Hardy-Weinberg proportions. The statistical significance of F_{IS} and thereby deviation from random-mating is tested by using a χ^2-test equal to $F_{IS}N_i$ with one degree of freedom, N_i is the sample size (*e.g.* Craddock *et al.* 1995).

F_{ST} is estimated as the mean of the standardised variance over the alleles. Testing of deviation from zero, *i.e.* no differentiation among the populations, can be done by using a χ^2-test equal to $2NF_{ST}$ with n-1 degree of freedom, N is the total sample size and n is number of samples.

From the estimated value of F_{ST}, the gene flow can be calculated as

$$Nm = \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$$

where Nm is the average number of migrants per generation.

Estimation procedures for F_{ST} from molecular markers can be done as described by Wright (1951), as θ (Weir & Cockerham 1984) or as G_{ST} (Nei & Chesser 1983). Nowadays, several computer programs are available for doing the estimations, *e.g.* BIOSYS (Swofford & Selander 1989), GENEPOP (Raymond & Rousset 1995) POPGENE (Yeh *et al.* 1999) and G-stat (Siegismund 1995).

The estimate Nm may be used for estimating the effective population size by assum-

ing, that the genetic identity I is a function the migration rate m and mutation rate ν as shown below

$$I = \frac{m}{m+\nu}$$

as described by Nei (1987). If ν is assumed to be about 10^{-6} m can be estimated and applied to the expression for Nm and thereby a rough estimate for N_e may be obtained.

Another method is based on the existence of private alleles, which means that a particular allele only found in one of the subpopulations. The method was developed by Slatkin (1981; 1985b; 1993) and a comparison between the F_{ST} and private alleles method was done by Slatkin & Barton (1989). The private alleles method utilises the average frequency of private alleles p(1) found in only one single subpopulation and is related to Nm as shown below

$$\ln(\bar{p}(1)) = a \ln(Nm) + b$$

where a and b are constants, depending on the sample size (Barton & Slatkin 1986).

Finally, it ought to be mentioned that estimation of genetic distances and dendrograms may be helpful for understanding the gene flow. This can support the hypotheses about how dispersal and interactions among populations are taking place.

APPLICATION

Effective population size

The method using the temporal variation in allelic frequencies have been used to some extent for marine molluscs. Table 1 shows a list on the species to which is has been applied and the references.

Allcock *et al.* (1997) have used the combined method by using the estimate for Nm and for genetic identity I to estimate the effective population size of the Antarctic octopus *Pareledone turqueti*.

Regarding the method using linkage disequilibrium the search in various databases did not reveal any investigations on marine molluscs, where this method has been applied. However, some references on fish are available (*e.g.* Bartley *et al.* 1992).

Table 1. List on marine molluscs where effective population size has been estimated and the references

Species	Reference
Crassostrea gigas	Hedgecock & Sly 1990
Crassostrea gigas	Hedgecock *et al.* 1992
Crassostrea sikamea	Hedgecock *et al.* 1993
Crassostrea virginica	Hedgecock *et al.* 1992
Haliotis tuberculata	Mgaya *et al.* 1995
Mercenaria mercenaria	Hedgecock *et al.* 1992
Penaeus japonicus	Hedgecock *et al.* 1992
Pinctada martensii	Hedgecock *et al.* 1992

Gene flow

As mentioned previously, the application of F_{ST} is the most widely applied method for describing differentiation among subpopulations. However, the genetic identity *I* also indicates the differentiation. Estimation of *I* and depiction of dendrograms by various methods give a hint about how the divergence has been established, and many studies have compared *I* with the geographic distances by application of Mantel's test (Manly 1985). However, the application of F_{ST} is obvious from the number of studies shown in Table 2. In Table 2, both the estimation procedures according Wright (1951) and Weir & Cockerham (1984) have been used. Table 3 shows studies where G_{ST} has been used. Table 4 lists species for which private alleles have been used (Slatkin 1985b). It has to be emphasised that none of the lists is exhaustive.

Several studies have applied both the estimation of F_{ST} and G_{ST} and furthermore used the method for private alleles. The estimates obtained by the various methods are mainly of the same magnitude (*e.g.* Brown 1991; De Wolf *et al.* 1998). The comparison between the methods for estimation of gene flow has been done by Bohonak (1999) and all the method were to some extent corre-

Table 2. List on species for which F_{ST} has been estimated

Species	Reference
Adalaria proxima	Todd *et al.* 1998
Austrocochlea constricta	Parsons 1996
Bathymodiolus thermophilus	Craddock *et al.* 1995
Bembicium vittatum	Johnson & Black 1991,
Bembicium vittatum	Parsons 1996
Branchinecta sandiegonensis	Davies *et al.* 1997
Bullia digitalis	Grant & da Silva-Tatley 1997
Calyptogena magnifica	Karl *et al.* 1996
Cerastoderma edule	Hummel *et al.* 1994
Cerastoderma glaucum complex	Hummel *et al.* 1994
Crassostrea angulata	Michinina & Rebordinos 1997
Donax deltoides	Murray-Jones & Ayre 1997
Eulepetopsis vitrea	Craddock *et al.* 1997
Goniodoris nodosa	Todd *et al.* 1998
Haliotis rubra	Brown 1991
Lepetodrilus elevatus	Craddock *et al.* 1997
Lepetodrilus galriftensis	Craddock *et al.*. 1997
Lepetodrilus pustulosus	Craddock *et al.* 1997
Littorina mariae	Rolan-Alvarez *et al.* 1995
Littorina obtusata	Rolan-Alvarez *et al.* 1995
Littorina striata	De Wolf *et al.* 1998
Mytilus edulis	Corte-Real *et al.* 1994
Mytilus edulis	Quesada *et al.* 1998
Mytilus edulis	Tremblay *et al.* 1998
Mytilus galloprovincialis	Skalamera *et al.* 1999
Mytilus galloprovincialis	Quesada *et al.* 1998
Neritina granosa	Hodges & Allendorf 1998
Nucella lamellosa	Grant & Utter 1988
Nucella lapillus	Day *et al.* 1994
Ostrea edulis	Saavedra *et al.* 1993
Pareledone turqueti	Allcock *et al.* 1997
Perna perna	Grant *et al.* 1992
Stramonita (=Thais) haemastoma	Liu *et al.* 1991
Strombus gigas	Mitton *et al.* 1989
Synalpheus brooksi	Duffy 1996
Tridacna gigas	Benzie & Williams 1995
Tridacna maxima	Benzie & Williams 1997
Tridacna maxima	Kittiwattanawong 1997

Table 3. List on species for which G_{ST} has been estimated

Species	Reference
Bembicium vittatum	Johnson & Black 1996
Eulepetopsis vitrea	Craddock *et al.* 1997
Lepetodrilus elevatus	Craddock *et al.* 1997
Lepetodrilus galriftensis	Craddock *et al.* 1997
Lepetodrilus pustulosus	Craddock *et al.* 1997
Littoraria cingulata	Johnson & Black 1998
Littorina littorea	Johannesson 1992
Littorina mariae	Tatarenkov & Johannesson 1994
Littorina saxatilis	Johannesson *et al.* 1993
Littorina striata	De Wolf *et al.* 1998
Melarhaphe neritoides	Johannesson 1992
Mytilus galloprovincialis	Quesada *et al.* 1995
Nucella lamellosa	Grant & Utter 1988

Table 4. List on species for which private alleles have been used for estimation of gene flow

Species	Reference
Bullia digitalis	Grant & da Silva Tatley 1997
Haliotis rubra	Brown 1991
Littorina striata	De Wolf *et al.* 1998
Perna canaliculus	Gardner *et al.* 1996
Strombus gigas	Mitton *et al.* 1989

lated with the dispersal ability of organisms. Despite the fact that populations are exposed to many forces acting on these, the estimates for gene flow provide valuable information on the migration of individuals among the populations (Bohonak 1999). An excellent review on the reproductive mode and genetic differentiation has been presented by Kittiwattanawong (1999) in the previous conference. McQuaid (1996) has presented review on Littorinidae showing evidence for both correlation between heterozygosity and larval dispersal and also for no correlation, indicating that the under-

standing of population dynamics is not simple.

PRECAUTION

All the methods presented in this review are based on polymorphic loci and frequencies of the alleles. Recognition of the subject, polymorphic loci, is the fundament for doing the estimate of effective population size and of gene flow. Any scientist, who has worked with allozyme electrophoresis or with polymerase chain reaction (PCR) and analysis of these products, is quite well aware of that just a slight change in the analytic condition may reveal or hide a variation. So, the first precaution is that the scientists dealing with these topics have to put effort so much as possible in obtaining the most efficient procedure. However, the financial support very often is limiting the effort, which can be utilised for establishing the optimal analytic procedures. A question, commonly asked, is how many loci should be studied. The answer is as many as possible, because the more loci the better information on gene flow and introgression of genes from other populations is obtained. Again, a limiting factor is the financial support, but also the history of the species has an impact on the variation found. If the population has passed a bottleneck recently, the genetic variation within the population may be low or if the mating success of the population is restricted to rather few individuals (*e.g.* Chakraborty & Leimar 1987). Finally, the reliability of the frequencies has to be considered. The relation between allelic frequency and the number of individuals scored is depicted in Figure 2.

The deviation of the allelic frequency is reduced with about 50%, when analysing around 50 individuals, so around 50 to 70 individuals will be an appropriate sample size to obtain a reliable estimate of effective population size or gene flow.

CONCLUSION

The application of molecular markers for estimation of effective population size and/or gene flow is a versatile tool and can be used as supplement for understanding the population dynamics. The estimates are profoundly dependent on the molecular markers used and the reliability of the analytical method and have to be considered as a supplement to other methods, *e.g.* mark-recapture and consensus experiments.

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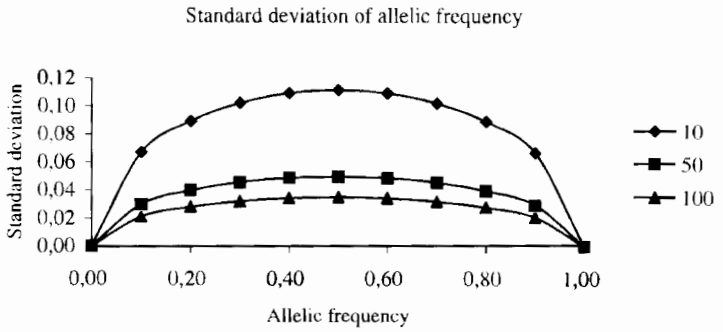

Figure 2. Standard deviation of allelic frequency as a function of allelic frequency based on various numbers of individuals (10, 50 and 100).

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