



Genetic adaptation to brackish-water environments

Brackish water
Lagoons
Genetic adaptation
Polymorphism
Ecological genetics
Eaux saumâtres
Lagunes
Adaptation génétique
Polymorphisme
Génétique écologique

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ABSTRACT

A number of possible mechanisms of genetic adaptation to brackish water, with particular reference to lagoon environments, have been considered. Several examples refer to experiments carried out on copepods of the genus *Tisbe*, mainly from the lagoon of Venice. Cases of natural genetic polymorphisms controlling the adaptation to salinity and temperature are reported. A reduced degree of visible polymorphism in lagoon populations has been observed in some species of copepods and isopods. A similar decrease of the genetic variability emerges from studies in which other kinds of genes have been examined: namely the loci controlling allozymic variation and those determining inbreeding depression. Two genetic strategies appear to be more likely to endow brackish-water species with the adaptive mechanisms suitable to face these severe environments. It is suggested that: A) the genes producing a high degree of individual homeostasis might be selected for, eventually reaching fixation with a loss of genetic variability; and B) a gene duplication might determine the fixation of the heterozygote condition, without loss of unfit genotypes, when such a condition produces the fittest phenotype. Some applied aspects of these genetic investigations are briefly discussed.

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RÉSUMÉ

Adaptation génétique aux milieux saumâtres.

Plusieurs mécanismes d'adaptation génétique aux milieux saumâtres lagunaires sont analysés. Ils sont illustrés par des expériences effectuées sur des copépodes du genre *Tisbe* provenant pour la plupart de la lagune de Venise. On montre, par exemple, que des polymorphismes génétiques sont à l'origine d'une adaptation à des régimes variables de salinité et de température. On a observé souvent une réduction du degré de polymorphisme chez des populations lagunaires d'un certain nombre de crustacés (copépodes, isopodes essentiellement). Une diminution semblable de la variabilité génétique a été mise en évidence pour des gènes différents: d'une part, ceux qui contrôlent la variabilité alloenzymatique et, d'autre part, ceux qui déterminent une réduction de la survie par « inbreeding ». Deux stratégies génétiques peuvent être suggérées pour expliquer les mécanismes adaptatifs qui permettent aux espèces d'eau saumâtre la survie dans ce milieu très sélectif. En premier lieu, les gènes qui déterminent un degré d'homéostasie élevé peuvent être fixés ce qui entraîne une diminution de la variabilité; en second lieu, une duplication génique pourrait fixer un état hétérozygote, sans perte d'allèles, dans le cas où l'hétérozygote produit un phénotype particulièrement bien adapté. Certaines implications pratiques de ces travaux sont également discutées.

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INTRODUCTION

The great majority of brackish-water habitats are areas of relatively small extension, situated where marine waters come into contact with fresh waters of continental origin. This is the typical condition in estuaries, deltas and brackish

coastal lagoons, which are characterized — even within short distances — by gradients for the main environmental parameters.

A further, and perhaps more important feature, is the presence in certain points of more or less widely fluctuating conditions, with short or very short periods and, often,

varying in an unpredictable way. This applies to salinity or, more generally, to the chemical composition of the waters, and also to physical factors such as temperature which may undergo remarkable changes in time and space, especially in shallow waters.

Much is known about the great physiological homeostasis achieved by the inhabitants of the variable brackish water environment (Kinne, 1964; 1971). However the cases of homeostatic adaptation in the physiology of populations or subspecies known to be genetic are very few. We believe that this is merely due to the paucity of marine forms which have so far proved suitable for genetic studies.

For the study of inherited physiological differences, certain harpacticoid copepods belonging to the genus *Tisbe* offer excellent material. One of the earlier experiments on these organisms dealt with three geographic populations of *T. holothuriae* (from Plymouth, UK; Chioggia and Gargano, Adriatic Sea) which have been compared as to their tolerance of diluted sea water (Battaglia, D'Avella, 1964). After a two year acclimation period to standard laboratory conditions at a salinity of 34‰ and at a temperature of 18°C, animals were exposed to a salinity of 18‰ and the times for recovery from osmotic shock recorded. The observed differences, related to the average salinity of the places where the populations came from, and the greater tolerance shown by the F₁ hybrids between Plymouth and Gargano, suggested that the differential tolerance to salinity variations was due to genetic factors.

Following this early experiment, further research on genetic adaptation to brackish waters was carried out, initially in the lagoon of Venice. The results are summarized in the present paper, together with a tentative analysis of the mechanisms and strategies involved.

STUDIES ON GENETIC ADAPTATION CONDUCTED IN THE LAGOON OF VENICE

The lagoon of Venice, as well as the other ones situated along the North Adriatic coast, has polyhaline waters and most of its inhabitants are of marine origin. However, due to the great variability of its conditions in time and space, few marine forms are able to settle there permanently. A first selection occurs the moment the marine species enter the lagoon. Relatively few of them are able to survive in the new environment and therefore the lagoon, compared with the adjacent sea, is inhabited by a reduced number of species. Crustaceans, molluscs, echinoderms, fishes, and other organisms provide good evidence of this situation (Soljan, 1948; Giordani-Soika, 1948; Vatova, 1950; D'Ancona, Battaglia, 1962).

A strong selection takes place also inside the lagoon. This is shown especially by some sedentary forms, such as certain barnacles and polychaete worms, which undergo a gradual decrease, both in number of species and individuals, as they approach the inner parts of the lagoon (Rigo, 1942; Cognetti, 1958).

Natural selection in the lagoon may also act at an intraspecific level. Research aiming to detect the survival in the lagoon of those genotypes which possess the necessary abilities, was carried out utilizing some benthic copepods exhibiting adaptive genetic polymorphisms.

A selective effect of temperature has been found in the polymorphic species *Tisbe reticulata* both for natural and experimental populations. The allele determining the phenotype "maculata" (V^M) is favoured at lower temperatures (Battaglia, Lazzaretto, 1967).

The balanced polymorphism exhibited by *T. reticulata* appeared to be adaptive also with respect to salinity. Populations from Roscoff and from the lagoon of Venice showed a different response to this environmental parameter, and this accounts for the different frequencies with which the phenotype « violacea » was recorded in the two localities (Battaglia, 1957). It appears that the different abilities of the homozygotes « violacea » and « trifasciata »

to stand diluted sea water are due to different osmoregulatory powers. The two genotypes exhibit a significantly different uptake of ²²Na, ⁴²K, and ¹³⁷Cs, which indicates the physiological importance of these genetic differences (Battaglia, Bryan, 1964).

BRACKISH WATER ENVIRONMENT AND THE GENETIC STRUCTURE OF POPULATIONS

Another aspect of the relation between environment and the genetic structure of populations, which might be studied in brackish waters, is the degree of polymorphism in marine and brackish habitats.

From many observations carried out on species exhibiting visible polymorphisms, such as the copepods *Tisbe reticulata* and *Porcellidium fimbriatum*, and the isopod *Sphaeroma serratum* (Battaglia, 1954; 1964), it has been found that in the lagoon of Venice these species are represented by fewer forms than in typical marine habitats. This suggests that the same mechanisms which control the establishment of different species in the lagoon operate also at an intraspecific level. The reduced degree of polymorphism observed in the lagoon populations could be ascribed to the smaller number of niches offered by this environment compared to marine ecosystems. Another possible factor is the exacting nature of the lagoon environment where the chances of survival depend largely on the ability to tolerate the inconstancy of the medium.

However, a reduced degree of visible polymorphism does not necessarily imply a drop in genetic variability, since other kinds of genetic variability enabling the species to confront unpredictable environmental changes could be present in brackish populations. Therefore, we investigated if, in *Tisbe* and other organisms, there are other possible sources of genotypical plasticity which can endow the species, or the population, with potential adaptability.

A group of genes, which are probably different from those controlling the polymorphisms discussed so far, and which may be utilized as well to estimate the amount of genetic variability in natural populations, is provided by the loci responsible for biochemical polymorphisms. The recent development of an electrophoretic technique suitable for small animals (Bisoli *et al.*, 1976), has permitted the analysis of this kind of genetic variation in some marine and brackish-water populations of *Tisbe holothuriae* (Battaglia *et al.*, 1978 b).

Average heterozygosities, average numbers of alleles per locus, and percentages of polymorphic loci — i.e. the usual measures of genetic variability — were found to be lower in the lagoon populations of Sigeon than in the marine population of Banyuls-sur-Mer (Southern France). The two populations compared were sampled in localities ecologically different but geographically very close to each other. Further comparisons of the same kind, carried out on another species of *Tisbe*, namely *T. bulbisetosa*, showed a very similar trend. For this species too, the values of genetic variability decreased from marine to brackish-water populations (Bisoli *et al.*, 1979).

In *T. holothuriae* and *T. bulbisetosa* it also has been possible to estimate the « concealed genetic variability », detectable by analysing the effects of inbreeding depression, as suggested by Morton *et al.* (1956). A measure of genetic variability, expressed as the number of lethal equivalents present in a population, is obtained by relating the reduction of survival following consanguineous matings to the corresponding coefficients of inbreeding.

For *T. holothuriae*, marine populations were sampled in Banyuls-sur-Mer and Split (Eastern Adriatic); lagoon populations were sampled in Sigeon, Malamocco (lagoon of Venice), and Grado (lagoon of Grado-Marano, Upper Adriatic). Both in the Western Mediterranean and in the Adriatic Sea, the degree of concealed genetic variability proved lower for the populations inhabiting brackish water habitats as compared with those living in typically marine

conditions (Lazzaretto-Colombera *et al.*, 1976 ; Fava *et al.*, 1976 ; Battaglia *et al.*, 1978 a).

Moreover, a population of *T. bulbisetosa* was sampled close to the sea in the lagoon of Grado, a second one in the inner lagoon of Venice, and a third one in the lagoon of Scardovari (delta of the Po river) characterized by extreme fluctuations of environmental factors. Once again, the concealed genetic variability was shown to decrease from the nearly marine (Grado) to more typical brackish habitats (Cervelli, Fava, 1980).

An estimate of the genetic variability, by means of electrophoretic analysis, has been carried out on the mussel, *Mytilus galloprovincialis*, by comparing some populations from the lagoon of Venice with a marine one in the neighbouring Adriatic Sea. Although a gradient of pollution was superimposed, the data showed a reduced genetic variability for the populations settled in the most inner part of the lagoon (Battaglia *et al.*, 1980 a).

The hypothesis that there is a correlation between reduced genetic variability and environmental instability, has been tested also by including another very unstable, challenging environment such as that provided by rock pools.

A common inhabitant of this peculiar environment is the harpacticoid copepod *Tigriopus*. Although *Tigriopus* species cannot be considered brackish water copepods, they share with the latter several features. In some way, and depending on the season, a rock pool could be viewed as a most unstable brackish environment. In fact, during the periods of variable weather conditions, the alternation of rainy and sunny days determines a sequence of rapid, extreme fluctuations both for salinity and temperature. Many individuals, during their own life span, have to face repeatedly such changes.

Very low estimates of genetic variability were obtained studying allozymic variations in two species: *Tigriopus fulvus*, from Italy, and *T. brevicornis*, from Scotland (Battaglia *et al.*, 1978 b). These findings cannot be considered as conclusive, nevertheless they clearly indicate that the possession of a high level of genetic variability is not a necessary prerequisite for an organism to colonize successfully a highly variable, unpredictable environment.

STRATEGIES OF ADAPTATION

The cases reported above suggest that the organisms which live in brackish water environments must possess a high physiological flexibility in order to occupy such a rigorous habitat.

Three mechanisms, or genetic strategies, may be conceived to account for the acquisition of such versatility: strategies which, however, are not mutually exclusive. The first one is based on the fact that the strong selective pressure operating in these environments, very unstable and unpredictable, may permit the survival of only those organisms endowed with a sufficient degree of genetic variability enabling them to cope with the instability of the environment. Under this model, the populations undergo a severe balanced genetic load. Not a single case reported by us agrees with this hypothesis. There is, in fact, in these habitats, a clear trend towards a decrease of genetic variability.

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The second strategy is based on the fixation of those genes which confer a high degree of individual homeostasis. Such an adaptive strategy involves a loss of genetic variability. The data we have produced support this hypothesis: on the other side, it should be considered that the choice of the strategy depends to a large extent on the kind and length of the life cycle of the species concerned; that is, on the way each species perceives the « environmental grain ».

A third possible strategy, which however requires experimental support, might consist in a gene duplication leading to the fixation of the heterozygote condition, without the loss of unfit genotypes imposed at every generation (Fava, 1981). A mechanism of this kind, mediated by regulatory genes, could perhaps be at the base of the so called « non-genetic » adaptation.

All the possible mechanisms are now being explored experimentally in our laboratory.

SOME APPLIED ASPECTS

Thus far, we have mainly been dealing with certain theoretical aspects of adaptation to the brackish-water environment.

However, some practical aspects of this kind of research have to be mentioned.

The study of the genetic structure of marine and brackish water populations, and of levels of genetic variability, provides good opportunities for monitoring the genetic effects of certain artificial environmental factors such as, for instance, water pollution.

The knowledge of the reactions that man-induced perturbations produce on brackish-water species is particularly important because we are dealing with organisms which can be considered physiologically and, as we have seen, genetically stressed.

Our research group is involved in this topic both with field estimations of the effects of pollution on the genetic variability of natural populations (Battaglia *et al.*, 1980 a) and with laboratory experiments. These are mainly done with experimental populations of *Tisbe* that are exposed to a series of conditions which simulate the situation that can be encountered in the lagoon of Venice. The path of gene frequencies in a number of subsequent generations is recorded to estimate the potential modifications of the genetic structure of populations (Battaglia *et al.*, 1980 b). Effects of this kind are more subtle and less obvious than the short term effects on individual organisms; but they can be at least equally important, since they may affect, in the long run, the genetic make up of populations and consequently, in some cases, their survival.

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