COLLOQUE INTERNATIONAL DE BIOLOGIE MARINE
STATION BIOLOGIQUE DE ROSCOFF
(27 juin-4 juillet 1956).

GENETICAL AND MORPHOLOGICAL VARIATION IN *PURPURA LAPILLUS* WITH RESPECT TO LOCAL AND REGIONAL DIFFERENTIATION OF POPULATION GROUPS

By H. STAIGER
(Station Biologique de Roscoff).

Genetical heterogeneity is known to be among the most fundamental features of animal populations in terrestrial species. Heterogeneity, and its consequence, genetical polymorphism, is in fact of such a common occurrence that it may be regarded as being the normal condition of natural populations. In marine species, however, the evidence for heterogeneity is actually very scarce. There are some littoral species of Isopods (1, 2) and some Copepods (3) which exhibit a phenotypical polymorphism for coloration patterns (polychromatism), which has been found in some instances to have a genetical basis.

Genetical heterogeneity in marine animals may not be supposed to have consequences fundamentally different from those in terrestrial species; however, the relation between genetical population structure and environment conditions may find, in littoral species, a much clearer expression on a microgeographical level due to the extremely strong ecological clines on the coast. The conditions for genetical differentiation of populations are most favourable in intertidal species with a non-pelagic development; these conditions will necessarily lead to the establishment of colonies or of populations which present a high degree of genetical isolation. Isolation is in fact often fulfilled in a region with an extended tide range where habitats for a given species are separated from each other by stretches which do not suit its ecological requirements, and which may therefore prevent or strongly reduce exchange of genetic material between different populations. When assuming that the genetical equilibrium within a population is related to its particular habitat conditions, widely different genetical constitutions may be expected in separated populations on a local (microgeographical) as well as on a geographical scale.

(1, 2) Bocquet, Lévi and Teissier, 1951 ; Bocquet, 1953.
(3) Bocquet, 1951.

The extent of such local and regional diversification may amply be demonstrated in the intertidal prosobranch species *Purpura lapillus* (synonyms: *Nucella lapillus*, *Thais lapillus*). The genetical heterogeneity of *Purpura lapillus* has a chromosomal, *i. e.* structural basis. The species is differentiated into two forms which show widely different chromosome numbers; their haploid numbers are 13 and 18 (4). The difference of numerical chromosome constitutions is seen from the metaphases of the first maturation division in female meiosis which show 13 and 18 bivalent chromosome configurations, respectively, in the two chromosome forms.

The analysis of the numerical dimorphism is made possible by the fact that the two forms are fully interfertile, and that their chromosomes may be combined in any possible combination to diploid constitutions. The relation which links the two forms is of the kind that is generally called Robertsonian. The haploid set of form 13 contains 5 metacentric (V-shaped) chromosomes each of which corresponds to one pair of acrocentric (rod-shaped) chromosomes in form 18. Each of the 10 acrocentric chromosomes is partly or totally homologous to one arm of the corresponding metacentric element of the opposite chromosome form. The remaining 8 chromosomes are numerically identical in the two forms. The haploid chromosome sets are thus $8 + 5 = 13$ in form 13, and $8 + 5 \times 2 = 18$ in form 18.

The numerical dimorphism within the species *Purpura lapillus* is thus due to the 5 acrocentric-metacentric chromosome groups. This is the basis of the genetical variation system of the species, and at the same time the origin of diversification between populations on the local and the regional scale. Chromosomal polymorphism is due to the fact that, within each of the 5 chromosome groups responsible for the numerical dimorphism, heterozygosity for acrocentric and metacentric elements may freely be realized, as well as homozygosity for the ones or for the other. This permits the formation of a great number of chromosome constitutions; in fact, there are 243 different possibilities of combinations of these chromosomal elements to diploid constitutions, and even several thousands if other structural differences between the two haploid sets are equally accounted for. As judged from the chromosomal analysis of the populations, all of these constitutions may become realized. This may illustrate the meaning of the term " chromosomal polymorphism ".

The analysis of heterogeneity of populations with respect to chromosomes is based on the analysis of heterogeneous constitutions. Due to the numerical differentiation, it is possible, in each heterogeneous female individual, to determine the respective numbers of chromosomes originating from the two forms 13 and 18; these numbers, the sum of which is 10 in diploid constitutions, lie necessarily between the limits 10 acrocentric pairs + 0 metacentric chromosomes on one hand, and 10 metacentric elements + 0 acrocentric pairs on the other. The records of heterogeneous individuals then permit us to define the state of heterogeneity of a population with respect to the chromo-

(4) STAIGER, 1954.

somes of the two forms. A rough measure of heterogeneity, within a population polymorphic for chromosome constitutions, is obtained by taking the mean percentage of acrocentric chromosome pairs recorded in a statistical number of individuals from the same locality, and that of the metacentric chromosomes of the same individuals. The microgeographical distribution of the two forms and of the two chromosome structures may then be recorded.

The principle of microgeographical distribution is quite simple: form 13 occupies exposed places; form 18 is found on sheltered places (4 *bis*). The pure forms 13 and 18 are ecologically differentiated. The same principle holds for the degree of heterogeneity, *i. e.* the respective percentages of the chromosomes originating from the two forms. The extreme habitat conditions " exposed " and " sheltered " are linked by a whole series of intermediate conditions; such intermediate habitats are occupied by chromosomally heterogeneous populations, whereas those on extreme habitats are pure for the forms 13 or 18. The chromosomal composition of any colony of *Purpura lapillus* corresponds to the particular degree of exposure of its habitat, *i. e.* to the whole complex of environment conditions that are normally associated to exposure. Some populations of the region of Roscoff and of the bay of Morlaix are particularly illustrative in this respect. Thus, the highly exposed place Estellen bihan is entirely pure for form 13, *i. e.* this population contains 100 per cent metacentric chromosomes; there are still 96 per cent metacentrics at Les Bisayers, 92 per cent at Ile Blanche, 60 per cent at the intermediate locality Bloscon, 13 per cent at Pen Lann, and finally 1 per cent metacentric chromosomes only in the most sheltered place near the laboratory of Roscoff. In the same way, the complementary percentages of the acrocentric chomosomes of form 18 increase as the exposure decreases, from 0 per cent at Estellen bihan to 99 per cent at Roscoff. There exists thus a gradient for chromosome frequencies which runs parallel to a gradient for ecological conditions.

The principle that rules the microgeographical distribution of the two forms is of an ecological nature, and the same holds for the two types of chromosome structures which are involved in the dimorphism. It is inferred that the forms with 13 and with 18 chromosomes are genetically adapted to the corresponding habitat conditions " exposed " and " sheltered ", respectively. The adaptive character of the intraspecific dimorphism is further demonstrated by the distribution of the two forms on a larger geographical scale. If form 18 is really associated to sheltered habitat conditions, as it is suggested by the microgeographical situation, then it is to be expected that form 18 is absent from a coast which presents no sheltered places, and where consequently the ecological requirements of form 18 are not fulfilled. Its presence or absence in a given region would be governed by the structure of the coast.

From the point of view of coast structure, the region of Roscoff and of the bay of Morlaix is in a sharp contrast to the coast which extends to about

(4 *bis*) STAIGER 1954.

15 km. to the east of the latter between Primel and Locquirec-St. Efflam: the first region shows most varied environments, the intertidal zone is large, and sheltered areas are very extended at many localities; on the other hand, the second region is relatively uniform, the intertidal zone extremely narrow and predominantly exposed.

The distribution of the two forms corresponds entirely to this contrast of coast structure. In the Roscoff region, both forms 13 and 18 coexist side by side on their adequate habitats, and form heterogeneous colonies where they come into contact on intermediate places. In the second region Primel-Locquirec, however, the sheltered form 18 is missing; consequently, exposed localities as well as those of intermediate character are occupied by pure colonies of form 13, showing 100 per cent metacentric chromosomes. Acrocentric chromosomes of form 18 are missing in the *Purpura* populations between Primel and Locquirec.

The adaptive character of the chromosomal dimorphism is thus seen on two levels of distribution: local, and regional. The regional contrast implies at the same time a profound difference for chromosomal composition of populations on intermediate habitats: such populations are heterogeneous in the first region, but homogeneous for form 13 in the second region. The structure of the coast, by the ecological features involved and through adaptive properties of the chromosomes, determines the genetical variation system of the species on the basis of a pre-existing material of genetical variation.

The genetical differentiation of populations and of population groups may deeply affect the morphological characters of individuals and of populations. The quantitative traits of the shells of *Purpura lapillus* are strongly influenced by both the genetical structure of the population and the environmental conditions of its habitat. The regional differentiation for chromosomal composition of population groups then enables us to estimate the parts played by constitution and by environment in the realization of the phenotype. *Purpura lapillus* has been known for a long time to have an exceedingly high degree of morphological variation, *e. g.* for coloration and pattern, for size, stuntedness, surface structure of the shells and for shell thickness (5, 6). Among these, shell thickness is especially well suited to demonstrate the relation between chromosomal and morphological population structure, as well as the differences for morphological variation systems which depend on regional differences for chromosomal composition of population groups.

Shell thickness is statistically treated by the relative weight of the shells. The relative weight is computed by dividing the absolute weight through a volume index; this latter is obtained from the dimensions of the shells. Differences for the relative weight express in a fairly clear way differences for thickness of the shells, and especially so for the walls of the body whorl, as it may be seen directly from longitudinal sections of the shells.

(5, 6) COLTON, 1916, MOORE, 1936.

The population samples of the region of Roscoff show clearly that the relative weight of the shells as well as shell size (length) are related to the chromosomal composition of the population. The mean size shows two maxima in the pure populations of the forms 13 and 18; the means then decline as the heterogeneity increases and attain a minimum in a population of highest heterogeneity, *i. e.* with about equal percentages of acrocentric chromosome pairs and of metacentric chromosomes.—The relative weight exhibits a similar behaviour; in this case, the maximum mean is attained in the most heterogeneous population, and then gradually declines as the heterogeneity becomes lower towards the two pure form populations. Shell thickness is higher in heterogeneous than in pure form populations.

This holds first for population samples that are quite different for mean size; it is however easy to establish that the relative weight is independent from size. The size ranges of two populations of different chromosomal composition mostly overlap; individuals chosen in two such populations for identical size (size classes) then show significant differences for the relative weight in the same sense as the total populations do. This means that shells of identical dimensions have thicker body walls when heterogeneous for chromosomes than when homogeneous 13 or 18.—This relation between heterogeneity and shell thickness holds equally good for some regions on the south coast of Brittany where the two forms 13 and 18 coexist, and seems thus to be of general validity in the species.

Of particular interest for the comparison between different regions is the relation between shell thickness and exposure. Heterogeneity is related to exposure; this means that, when passing from intermediate to highly exposed places, the shell thickness declines as the degree of exposure increases.

This paradoxical relation between exposure and shell thickness is in fact a specific feature of heterogeneous population groups. In a region which is homogeneous for form 13, such as that to the east of the bay of Morlaix, the connexion between exposure and shell thickness is quite different. The degree of exposure which is attributed to any one colony of this homogeneous population group remains relative, but a comparison is possible between neighbouring colonies on a coast part which shows a clear gradient for exposure. Near Locquirec, in a semi-exposed situation due to some shelter afforded by a small islet off the land point of Locquirec, the mean relative weight is 0.365 ± 0.005 for a sample of about 80 individuals; on the northern exposed point of this islet, the mean is 0.420 ± 0.005; the increase is significant. In the same way, the means increase inside the bay of Locquirec, from 0.382 ± 0.004 at the semi-exposed locality Roches d'Argent to 0.404 ± 0.005 at the exposed place Plestin. In the heterogeneous population group of the bay of Morlaix, however, the means drop from 0.555 ± 0.003 (Bloscon) to 0.442 ± 0.004 (Estellen bihan) when passing from intermediate to exposed places, and where at the same time the heterogeneity declines from 60 per cent metacentrics-40 per cent acrocentric pairs at Bloscon to 100 per cent metacentrics at Estellen bihan.

Between intermediate and exposed, the shell thickness increases with increas-

ing exposure in a chromosomally homogeneous population group, but it declines in the same direction when this population group is heterogeneous for chromosomes. The shell thickness follows thus a morphological variation system that is fundamentally different in the two regions, according to presence or absence of chromosomal heterogeneity, and as a function of different genetical variation systems. This shows to what an extent the genetical background may intervene in determining the connexion between morphometric characters and environmental conditions—a fact which should particularly be emphasized in comparative regional studies. The genetical background is likely to be strongly different in separated regions. In *Purpura lapillus*, there exist, under certain habitat conditions, significant differences in the chromosomal composition of subcolonies at a distance of about 5 meters, and this even without any ecological barrier between the two places. These differences are quantitative, in that equilibria between different components of heterogeneity may become shifted from one place to the other. When passing to the geographical scale, qualitative differences may equally be expected, *i. e.* gene or chromosome mutations may take part in the heterogeneity on one place, and are absent from a geographically remote locality.

Genetical heterogeneity in terrestrial species is recognized to be concerned with the fitness of the populations (7), *i.e.* their capacity to withstand the varying environment conditions, and to exploit their habitats; it is therefore of particular interest to compare heterogeneous and homogeneous regions with respect to traits that are related to fitness. It is rather difficult to understand from this point of view the thickening of the shells in heterogeneous populations, since quite thinner shells may apparently respond to the demands of the environment, *i. e.* to even considerably higher degrees of exposure. There are however other differences of another nature to be observed between the two population groups in question. It is unknown at present whether or not such differences are associated to the regional differenciation for chromosomal population structure, but the contrasts are striking enough and deserve special attention.

One of these differences concerns population density. The density of *Purpura* populations on exposed localities with rich food supply is considerably higher in the heterogeneous region Roscoff-bay of Morlaix than on similar habitats of the homogeneous region Primel-Locquirec. A striking fact to be observed in this latter region is the existence of exposed points which are completely free from *Purpura*, although they would present a very rich food supply for them by carrying a dense mussel bed; comparable places in the bay of Morlaix show a density of several hundreds of *Purpura* individuals per square meter.

As a second point, the body size is equally different in the two regions. As stated earlier, the mean body size increases from intermediate to exposed places in the heterogeneous population group. In the homogeneous region,

(7) LERNER, 1954.

however, the maximum body size is attained on intermediate localities with rich food supply, and is then seen to decline as the exposure increases; finally, *Purpura* is absent from the most exposed places, as it has already been mentioned.

Mean body size and population density give together a relative measure for production; they express the ability of the population as a whole to utilize the resources of the habitat. As judged from these two facts, it seems that a heterogeneous population group is more versatile in this respect. The ecological preference appears to be shifted (from intermediate to exposed); it is possible that heterogeneity leads to a wider tolerance and to an enlargement of the range of habitat conditions which the species is capable of exploiting. Regional differences of this kind deserve some emphasis from the genetical point of view; they possibly point to a general meaning of genetical heterogeneity for the biology of populations.

BIBLIOGRAPHY

BOCQUET (C.), LÉVI (C.) et TEISSIER (G.), 1951. — Recherches sur le polychromatisme de *Sphæroma serratum* (F.). *Arch. Zool. Exp.*, **87**, 245-298.

BOCQUET (C.), 1953. — Recherches sur le polymorphisme naturel des *Jaera marina* (Fabr.) (Isopodes Asellotes). *Arch. Zool. Exp.*, **90**, 187-450.

BOCQUET (C.), 1951. — Recherches sur *Tisbe* (= *Idyæa*) *reticulata* n. sp. *Arch. Zool. Exp.*, **87**, 335-416.

COLTON (H. S.), 1916. — On some varieties of *Thais lapillus* in the Mount Desert region, a study of individual ecology. *Proc. Acad. Nat. Sc.* (Philadelphia), **68**, 440-454.

LERNER (I. M.), 1954. — Genetic homeostasis. *John Wiley and Sons*, Inc. (New York).

MOORE (H. B.), 1936. — The biology of *Purpura lapillus*. I. Shell variation in relation to environment. *J. Mar. Biol. Assoc. U. K.*, **21**, 61-89.

STAIGER (H.), 1954. — Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Oekologie der Art. *Chromosoma*, **6**, 419-478.

Discussion

M. Bullock. — I don't suppose the following case can be put to you as a question but perhaps as a reason to come to California. It might be of interest because it concerns populations of gastropods showing phenotypic differences related to ecologic differences.

Acmæa digitalis, a high tide limpet living both on exposed points and in harbours, presents some morphological differentiation between these populations. We undertook to test whether there is any physiologic differentiation using the sensitive method of reciprocal transplantation and survival of marked individuals over some months. According to a student of mine who has made these studies, there is a difference in viability, the samples from inside a breakwater showing much more mortality when transplanted to the outside, than outside samples transplanted to the inside, relative to control transplants.

In another species, *Acmæa limatula*, Dr. SEGAL in our laboratory, has shown, in a paper now in press, the existence of physiological differences between high tide populations and low tide populations and their complete reversibility by transplantation. He has also shown morphological differences between them. But what

may be of special interest in connection with Dr. STAIGER's results is that a large fraction of the population, those in the upper parts of the range of distribution, develop mature gonads but apparently never spawn, so that they as well as the rest must be replaced from spawn from a fraction of the population.

I wonder if this might be occurring in *Purpura*? And I hope we will some day have results of transplanting large samples of the populations of *Purpura*.

M. Barigozzi. — May I ask Dr. STAIGER for some informations about the first stages of the meiosis?

M. Staiger. — Most of the stages of meiotic prophase of females are inaccessible for cytological examination, the eggs are in diakinesis at the moment of spawning. In some exceptional cases, diplotene configurations may be seen; these show clear chiasmas.

M. Prosser. — Experiments, both in laboratory and field, should indicate what stress is critical in the two chromosomal types. You implied two possible physiological differences, ability to withstand mechanical stress such as wave pressure, and feeding or ability to extract nutrients from the food. Are these the probable bases of selection or are there others which might be used experimentally?

M. Staiger. — Quality and quantity of food is different for the different types of habitats (mussels, barnacles, prosobranchs), and it is therefore possible that the two chromosome forms show different ability in utilizing different food resources, and that this contributes to fitness. It is however difficult to make one only factor of the environment responsible for the ecological preferences of the two forms – it is rather the whole complex of habitat conditions that acts selectively: food supply, algal vegetation, mechanical stress through the waves; most of these factors are more or less tightly related to exposure. Transplantation experiments could perhaps give further informations about the respective importance of these components; e. g. transplantation of heterogeneous individuals from an intermediate habitat to an intermediate pure colony with a rich food supply (mussels), or with poor food supply (barnacles).

M. Smith. — It is somewhat difficult to see how chromosomal heterogeneity *per se* results in greater fitness of a population, but do you consider it likely that one of the values of heterogeneity may lie in the fact that a heterogeneous population produces a more diverse progeny? The result of this would be an increased probability that in any given year, a sufficiently large fraction of the brood will survive even if conditions vary greatly from the normal. For example, heterogeneity would be an advantage if, in the Bay of Morlaix, great differences in salinity were to occur in different years, but in each year some large fraction of the young *Purpura* could survive.

M. Staiger. — It seems that the homogeneous population group is morphologically more variable between as well as within populations (higher coefficients of variation in colonies that live under critical conditions, as poor food supply) than the heterogeneous one; this could lead to the assumption that a higher proportion of individuals would be eliminated in highly variable populations for metric traits in case of sudden changes of the environment (salinity, temperature, etc.). It is conceivable, as you suggested, that heterogeneous populations could better withstand environmental stresses of this kind, especially in the young stages, because of their higher genetic variability which may lead to a better equilibrium between the internal and the external environment. Perhaps the higher morphological variability of the homogeneous populations might be a consequence of environmental stresses under which the animals suffered when young.