season in two successive years. For comparison wild mice from the neighbouring mainland were sampled at the same time.

Blood was obtained from the suborbital canthal sinus of trapped mice and the unharmed animals were released again into the population. The serum and erythrocytes were separated and frozen for later analysis. The proteins were separated by horizontal starch-gel electrophoresis. In the erythrocytes the systems studied were haemoglobin *Hbb* (Gluecksohn-Waelsch, Ranney & Siskin, 1957) and the erythrocyte peptidases *PepC* and *PepB* (Lewis & Truslove, 1969). In the serum two esterase loci were examined, the pre-albumin esterase designated *Es2* (Petras, 1963) and a post-albumin esterase *Es5* (Petras & Biddle, 1967). In the β -globulin fraction of mouse serum the transferrins *Trf* were studied.

Both the Skokholm and mainland mice were polymorphic at all six loci. In fact of the 15 alleles found in the mainland samples all but one, that of *Es2*^c, occurred on the island. Of the mainland mice 17.8% are heterozygous at these polymorphic loci compared with 25.3% of the island mice. Of interest is the fact that on Skokholm natural selection was seen to be acting on the variants at three loci. Heterosis or an excess of heterozygotes over that expected on the Hardy-Weinberg equilibrium was found to exist at the beginning of the breeding season (March) at the *Es2* locus and at the end of the breeding season (September) at the *Hbb* locus. The gene frequencies at these loci returned to the expected value in September at the *Es2* locus and in March at the *Hbb* locus. Thus powerful selection pressures are shown to operate at both ends of the breeding cycle. There was selection against mice carrying the *PepC*^c allele during the summer. These trends were repeated in both years of our study. Marked numerical fluctuation in population size occurs during the breeding season from about 200 animals at the beginning to about 2000 at the end. Neither genetical nor physiological investigation (Berry, Jakobson & Moore, 1969) has uncovered any factor that can be seen to contribute to this.

These studies support the assumption that polymorphism is a widespread and important phenomenon and that it is generally associated with and maintained by heterozygous advantage. Electrophoretic separation of proteins enables us to witness selection in operation and to observe and analyse its immediate effects. Investigation by this method at the gene level by using a sampling technique that does not harm the individual or interfere with his contribution to subsequent populations has become a powerful tool in ecological and population genetics. Gene frequencies have been determined in man (Harris, 1969) and in *Drosophila pseudoobscura*

(Lewontin & Hubby, 1966), but this is the first time that a population has been studied through several generations where the forces contributing to the gene frequencies found can be better assessed.

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The Role of Thiol Groups in the Multiple Forms of an Invertebrate Creatine Kinase

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The creatine kinase (EC 2.7.3.2) of the marine polychaete *Murphysa sanguinea* has been purified and found to consist of five electrophoretically distinct forms, none of which appears to be associated with particulate matter of the cell or to show organ-specific distribution (Fitzsimmons & Doherty 1970). Carbohydrate was found to be associated with the enzyme protein, but was removed by gel filtration in 0.1 M-KCl without affecting the enzyme activity or multiplicity. Only partial separation of the multiple forms has been achieved to date despite the wide variety of fractionation procedures tested. It has been found that rechromatography of a mixture of forms 3, 4 and 5 on DEAE-cellulose at pH 8.3 resulted in the recovery of 16% of the total protein as forms 1 and 2. Refractionation of forms 3, 4 and 5 by precipitation with 65% (v/v) ethanol in the presence of magnesium acetate failed to produce detectable amounts of forms 1 and 2. Forms 1 and 2 had a considerably lower specific activity than a mixture of forms 3, 4 and 5, but were activated by incubation with 0.25 M-dithiothreitol to the extent of 350%. Under the same conditions forms 3, 4 and 5 show only a 26% increase.

Immunological studies indicated a close relationship of all five forms of the enzyme. An apparent interconversion of the molecular forms detected by gel electrophoresis was achieved by incubation with dithiothreitol and with the oxidized form of this compound. Titration of the thiol groups of the different enzyme forms with 5,5'-dithiobis-(2-nitrobenzoic acid) showed that both the easily titratable thiol groups and the total thiol groups

measured after treatment of the protein with guanidine hydrochloride are reduced in the enzyme form 1 as compared with a mixture of forms 3, 4 and 5. Electrophoresis of alkylated enzyme forms on sodium dodecyl sulphate polyacrylamide gels led to a major protein band in each case with an approximate molecular weight of 15 000.

Our results indicate that stepwise oxidation of thiol groups results in the formation of multiple electrophoretically distinct forms of creatine kinase from this invertebrate source. Serious doubts must thus be raised on the use of crude extracts of invertebrates for the detection of 'isoenzymes' of creatine kinase.

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from the wall by trypsin or papain, and the cation-binding capacity of the wall was thereby decreased by 50%.

Yeast phosphomannan may have a similar role to teichoic acids of bacteria in cation-binding and transport. In fact the act of flocculation, phosphate groups on the surface of adjacent cells attract Ca^{2+} to form salt bridges.

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Cation-Binding by Yeast Cell Walls

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Brewer's-yeast cell walls were isolated from flocculent and non-flocculent strains. Samples (10mg) of cell wall were washed with 1mM-EDTA and suspended for 40 min at 25°C in 3.7mM-calcium chloride (0.4ml) containing 0.4mCi of ^{45}Ca at pH 4.5. The walls were sedimented at 2000g, washed with glass-distilled water (3 × 2ml) and dried, and the radioactivity was measured in a liquid-scintillation counter (Lutwak, 1959). Flocculent yeast walls bound on average 3.4mmol of Ca^{2+} /100g dry wt. of wall whereas non-flocculent walls bound 1.6mmol/100g dry wt. of wall.

Mg^{2+} and certain other cations replaced Ca^{2+} freely. The degree of binding with Ca^{2+} was affected by prior treatment with surface-acting agents, e.g. maltose and urea, or esterifying agents, e.g. 1,2-epoxypropane. Walls derived from yeast grown in an Mg^{2+} -deficient medium had a greater ability to bind cations and a higher phosphorus content than controls. [Growth of *Bacillus subtilis* in an Mg^{2+} -limited medium also led to an increase in cell-wall phosphorus (Tempest, Dicks & Ellwood, 1968).] Yeast grown in a phosphorus-deficient medium bound Ca^{2+} to a below-normal extent.

Flocculent cell walls fractionated with ethylenediamine (Korn & Northcote, 1960) had higher phosphorus contents in 'fraction A' than did non-flocculent walls (Lyons & Hough, 1970). Gel electrophoresis and gel electrofocussing revealed the presence in 'fraction A' of a phosphorylated manno-protein, involving phosphodiester linkages, with a low isoelectric point. This component was released

Some Properties of the Ion-Gating Polypeptide Alamethicin

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Physicochemical studies on solutions of the cyclic polypeptide alamethicin have been carried out (Chapman *et al.* 1969; A. I. McMullen & J. A. Stirrup, unpublished work) in an attempt to throw light on the relationship between its structure and its 'excitability-inducing' properties in phospholipid bilayers (Mueller & Rudin, 1968). Interfacial-tension studies have indicated that micelle formation occurs in aqueous solvents at a very low concentration, and ultracentrifuge experiments show that the aggregates increase in size as the concentration increases, at fixed ionic strength. The presence of 6M-urea decreases the size to about 5 monomers/micelle, and alcohols prevent aggregation. Increase in ionic strength increases the aggregation number, and the pH-titration curve indicates that the carboxyl group has a high pK_a value, exhibiting marked hysteresis in the alkaline region.

The 'pure' material can be separated by t.l.c. into three components of very similar, but not identical, amino acid contents (A. I. McMullen, J. H. R. Slade & J. A. Stirrup, unpublished work). Molecular models of the major component, which corresponds closely to the structure of alamethicin proposed by Payne, Jakes & Hartley (1970), indicate that no significant degree of parallel or antiparallel pleated sheet hydrogen-bonding is possible either inter- or intra-molecularly. This is due to the presence of the well-distributed methylalanine (and proline) residues. The presence of two methyl groups on the α -carbon of the former peptide prevents, by steric