BIOCHEMICAL GENETICS OF HYBRIDISATION IN EUROPEAN HOUSE MICE

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SUMMARY

Techniques for demonstrating allozymic variation in seven enzymes (four esterases, isocitrate dehydrogenase, malic enzyme, and malate dehydrogenase) were employed to study genic variation in a narrow zone of hybridisation between allopatric semispecies of the house mouse (*Mus musculus musculus* and *M. m. domesticus*) on the Jutland Peninsula of Denmark. Material consisted of 2696 mice collected at 152 farms representing 44 sample areas on the peninsula and adjacent islands. The history of movements of early farming cultures with which mice were associated as commensals suggests that *musculus* and *domesticus* have been in contact and hybridising in northern Europe since 3000 B.C. The zone in Jutland and another in Germany lie in regions transitional between Atlantic and continental climates, the two parental forms meeting where they are equally well adapted to ecological conditions.

The zone in Jutland has not shifted since Ursin defined its position in 1952 on the basis of morphological characters. An analysis of genotypic proportions in populations in the zone of hybridisation failed to demonstrate assortative mating, thus supporting laboratory evidence of free interbreeding between the semispecies. Genic heterozygosity levels are " normal " on the large islands of Falster, Fyn, and Als but reduced on the small islands of Alrø and Hjaelm, presumably through the founder effect or genetic drift. The zone is strongly asymmetrical north to south, with extensive introgression of *domesticus* alleles into *musculus*, but little introgression in the other direction. A marked increase in width of the zone in western Jutland is associated with a more extensive gradient of environmental factors, particularly precipitation. In the narrow eastern part of the zone, 90 per cent. of the transition in genetic character (as measured by a hybrid index) occurs over a distance of 20 km. The extent of introgression varies markedly among loci. Linkage between the loci studied is not a major factor affecting patterns of introgression. The extreme steepness of the gradient of transition in genetic character and the occurrence of major changes in frequencies at all loci along the same line are cited as evidence that the selective values of alleles are determined in part by the internal genetic environment. The "new" genetic environment created by introgression of *domesticus* alleles into *musculus* populations apparently favours the occurrence of minor alleles at the *Es-2* and *Es-3* loci. The failure of the *musculus* and *domesticus* gene pools to fuse despite long-standing hybridisation argues that genetic isolation cannot be equated with reproductive isolation. Selection against introgression of the genes studied (or the chromosomal segments that they mark) is presumed to involve reduced fitness in backcross generations caused by disruption of co-adapted parental gene complexes.

1. INTRODUCTION

THE remarkable narrowness and apparent temporal stability of many zones of allopatric hybridisation present an intriguing problem in evolutionary genetics. Mayr (1963, p. 378) has suggested that there is in such zones " a vigorous selection against the infiltration of genes from one balanced gene complex into the other, but without the development of any isolating mechan-

isms as a by-product of this selection ". Restriction of gene flow is thus attributed to an interdependence of genes and a consequent genetic imbalance between differentiated gene pools. While Huxley (1943), Mayr (1963), Bigelow (1965) and Ford (1971) maintain that zones of allopatric hybridisation often reach stable equilibrium, with introgression balanced by selection, Wilson (1965) and Remington (1968) believe that they are generally transitory, being eliminated with the perfection of reproductive isolating mechanisms or fusion of the parental gene pools. All hypotheses invoking hybrid inferiority predict that introgression involves something more than a simple diffusion of alleles (Mayr, 1963).

The present study of variation in allele frequencies at seven gene loci through a narrow zone of hybridisation between two allopatric forms of the house mouse (*Mus musculus musculus* and *M. m. domesticus*) on the Jutland Peninsula of Denmark was motivated by the following questions: (1) Is introgression occurring beyond the geographic limits detected in an earlier analysis of morphology (Ursin, 1952)? (2) What environmental, historical, or other factors are important in determining the position and width of the zone? (3) Do patterns of introgression vary among loci, and is introgression symmetrical? (4) Do patterns of introgression provide evidence relevant to the current controversy regarding the selective neutrality or non-neutrality of allozymic variants?

2. Systematic and ecological background

(i) *Differences between the parental forms*

European house mice were assigned to two subspecies groups by Schwarz and Schwarz (1943), the *spicilegus* group represented by *M. m. musculus* and *M. m. spicilegus*, and the *wagneri* group including *M. m. domesticus* and *M. m. brevirostris* (fig. 1). *Domesticus* and *musculus* hybridise on the Jutland Peninsula and on a front extending from Kiel Bay on the Baltic Sea south through Germany (Zimmermann, 1949). The positions of these disjunct zones roughly correspond to those of similar zones of hybridisation involving the carrion and hooded crows (*Corvus c. corone* and *C. c. corax*), as described by Meise (1928).

Morphological and ecological differences between *musculus* and *domesticus* were discussed by Zimmermann (1949) and Ursin (1952). *Musculus* is a short-tailed, light-bellied mouse with a brownish yellow lateral line, whereas *domesticus* is long-tailed and dark-bellied, with no obvious discontinuity of colour on the sides. *Domesticus* is slightly larger than *musculus* and has more tail rings.

Schwarz and Schwarz (1943, pp. 70-72) maintained that *domesticus* is a specialised commensal, while *musculus* has not progressed beyond " a primitive stage of commensalism ". But Ursin's (1952) and our field work in Denmark indicates that *musculus* is fully as successful as *domesticus* in exploiting farm buildings and other man-made " habitats ". Zimmermann (1949) reported that *musculus* is more resistant to cold and has a greater tendency to winter outdoors, but populations of either form rarely overwinter in fields in Denmark (Ursin, 1952). The long tail of *domesticus* was regarded by Schwarz and Schwarz as an adaptation for commensalism, but Zimmermann questioned this interpretation by demonstrating that variation in tail length in Europe follows Allen's rule.

In a study of the behaviour of laboratory-raised individuals of both forms and their F_1 hybrids, Brubaker (1970) found that *domesticus* showed greater wheel-running, swimming and " open field " activity, while *musculus* had a greater tendency to hoard. We have noted that wild-caught *domesticus* struggle vigorously and bite when handled, whereas *musculus* are relatively docile and infrequently bite. *Musculus* is also more active than *domesticus* in the daytime.

Musculus and *domesticus* differ more in appearance and behaviour than the average run of subspecies designated by taxonomists, and they are quite distinct genetically. From an analysis of allozymic variation in Jutland

Fig. 1.—European distribution of four subspecies of *Mus musculus*. Zones of allopatric hybridisation between *musculus* and *domesticus* are indicated by crosses.

populations of *musculus* and *domesticus*, Selander, Hunt and Yang (1969) derived a coefficient of genetic similarty of 0·79, a value close to those obtained for sibling species of the rodents *Sigmodon* and *Peromyscus* (Selander and Johnson, 1973). Considering the relatively strong differentiation of *musculus* and *domesticus* and, more importantly, the apparent isolation of their gene pools despite hybridisation (see Discussion), it is realistic to consider them semispecies rather than subspecies.

(ii) *Establishment of the zone of hybridisation*

Zimmermann (1949) suggested that *musculus* was derived from *spicilegus* and reached the Baltic Sea area during the post-glacial optimum, when several other dry-land vertebrates now represented in northern Europe by relic populations ranged north to the Baltic Sea. (House mice have been reported from an early post-glacial forest fauna in central Germany by

Brunner, 1941.)   Following its establishment in northern Europe, *musculus* is presumed to have changed from an entirely aboriginal to a partially commensal form and subsequently to have accompanied the spread of grain-farming into north-central France, establishing itself over much of northern Europe by 4200 B.C.

Domesticus is believed to have reached Europe later than *musculus*, arriving as a commensal associated with the neolithic grain culture that spread from North Africa into Spain and southern France (Zimmermann, 1949).   By 3000 B.C. the long-isolated farming traditions of the western Mediterranean and northern and eastern Europe made contact, and at this time it is likely that *domesticus* came to occupy Britain, northern France, and western Germany.   The probable position of the zone of contact of these cultures, as described by Waterbolk (1968), lies several hundred miles west of the present boundary between *domesticus* and *musculus*.   Because the European distribution of *musculus* corresponds to the drier " continental " climate, and that of *domesticus* to the wetter " Atlantic " climate, the shift in the zone of contact may have been caused by an eastward movement of the climatic boundary since 3000 B.C.   Alternatively, *domesticus* simply may have replaced populations of *musculus* that earlier had occupied the eastern margin of the Atlantic climatic region.   In any event, it is likely that interbreeding occurred when the two forms first met and that a zone of hybridisation has existed in some form in central-northern Europe for several thousand years.

The present disjunction of the northern Jutland and German populations of *musculus* presumably was established as the zone of contact moved eastward, thus permitting *domesticus* to occupy the southern Jutland Peninsula.   This invasion did not displace *musculus* from the larger islands in the western Baltic Sea, including Als and Fyn.

(iii)   *The environment of Jutland*

The Jutland Peninsula is 435 km. long and varies in width from 50 km. at the German border to 178 km. in the central region.   The topography is principally the result of Quaternary glaciation, and particularly the last glacial period, when ice sheets covered the northern and eastern parts (Davies, 1944).   Ice emanating from Scandinavia retreated earlier and more rapidly than that which had spread westward from the Baltic.   Consequently, eastern Jutland has a greater and more varied relief than northern Jutland because of the longer period available for the accumulation of morainic deposits.   In northern Jutland the soil is mainly sandy loam of relatively low fertility, and the terrain is bare and windswept.   The landscape of eastern Jutland is characterised by numerous valleys and rounded hills (up to 172 m. in elevation), and soils are mainly clay loam and sandy loam.   Because glacial drainage flowed westward, western Jutland is a flat, sandy outwash plain with scattered low sandhills formed in the second glacial period.   In geology and landscape, the Danish islands are similar to eastern Jutland.

Because of the small size and gentle relief of Jutland, there are no large rivers.   Many small lakes occur on the peninsula, and there is a large estuary, the Ringkøbing Fjord, in central-eastern Jutland.   The northern and central regions are separated by the Limfjord, a waterway extending from the North Sea to the Baltic Sea.

The climate of Jutland and adjacent islands is fairly uniform.   However, mean yearly precipitation decreases from south to north, and the transition

occurs much more gradually in western than in eastern Jutland (fig. 2). The lowest temperatures occur inland in the central and northern half of the peninsula, but the actual range of geographic variation in temperature is very small.
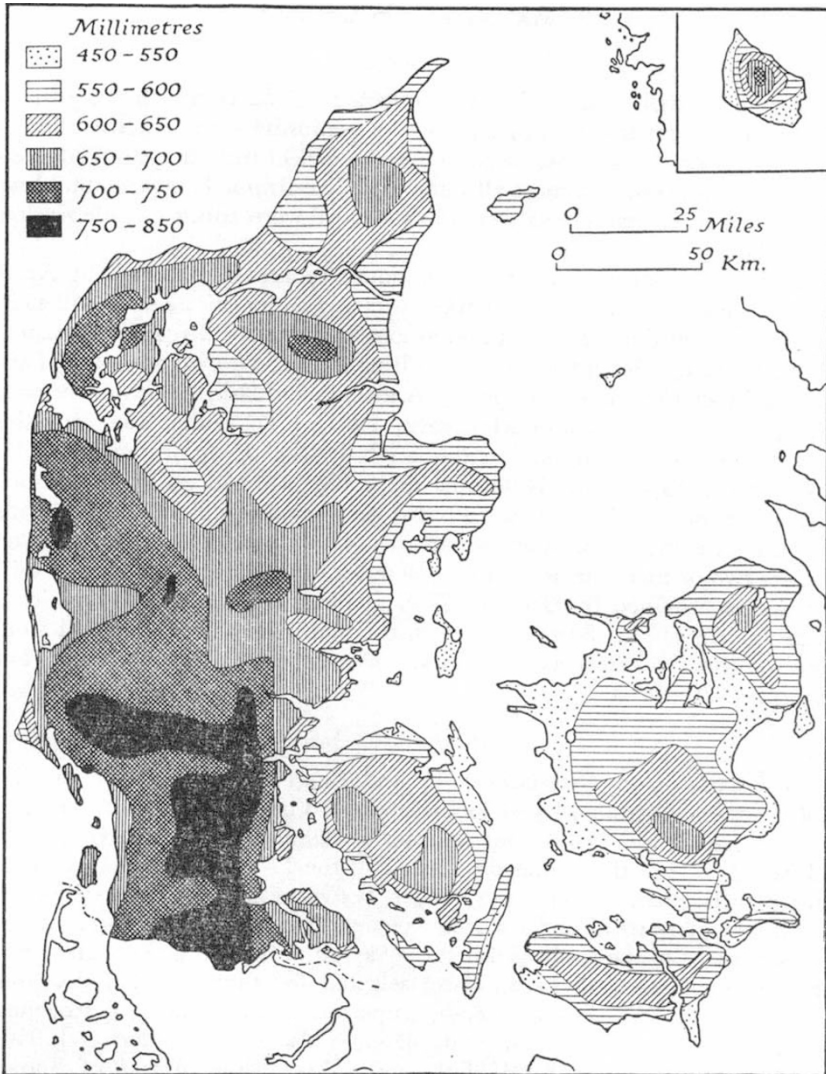

Fɪɢ. 2.—Total yearly precipitation in Denmark. From Davies (1944) after Danske Meteorol. Inst. (1933, plate 40).

The distribution of native vegetation and agricultural crops (predominantly cereals) corresponds fairly well to that of soil types. Eastern Jutland and the islands have been under cultivation for centuries, with forest largely confined to the higher land and steeper slopes. Before 1800 large regions of sandy soil in western and central Jutland were covered by a native heath. Although farming has increased in the west and north in the last 100 years,

the density of towns and farms has remained much lower in these regions than in the east. Agriculture is now very uniform throughout Denmark; approximately four-fifths of the land area is agricultural, and of this more than 80 per cent. is arable (Davies, 1944).

3. MATERIALS AND METHODS

(i) *Samples*

A total of 2696 house mice was collected at 152 farms on the Jutland Peninsula and on five islands east of the peninsula from August 1968 to February 1969 (fig. 3). Because attempts to collect mice in grainfields were generally unsuccessful, almost all mice were live-trapped in farm buildings or captured when grain ricks were threshed. All were adults or independent subadults.

Studies of allozymic variation in house mouse populations in North America have demonstrated marked heterogeneity in allele frequencies among barns on the same farm and among farms in local regions (Selander and Yang, 1969; Selander, 1970), resulting from the polygynous, tribal and territorial social system of the species (Anderson and Hill, 1965; Reimer and Petras, 1967; DeFries and McClearn, 1972). To reduce the confusing effect of local differentiation in our analysis of geographic variation, the farms were grouped into 44 sample areas (fig. 3), and estimates of allele frequencies for sample areas were based on pooled samples from all farms. With three exceptions (insular sample areas 42, 43 and 44), each sample area includes two or more farms. (A complete list of farms and numbers of mice collected is provided by Hunt [1970].) Farms were coded alphabetically within sample areas. Sample areas on the mainland were numbered along three south-north transects as follows: eastern, 1-16; central, 17-30; and western, 31-39 (fig. 3).

(ii) *Allozymic analysis*

In an earlier study (Selander, Hunt and Yang, 1969), allozymic variation in 36 proteins controlled by 41 loci was assessed in two samples of *domesticus* from southern Jutland and four samples of *musculus* from northern Jutland and islands east of the peninsula. At 13 of the 17 variable loci there were marked differences in allele frequencies between the semispecies; and at several loci alternate alleles were fixed or nearly so in northern and southern populations. On the basis of this survey seven polymorphic enzymes (each encoded by a separate locus) were selected for detailed analysis: four esterases (*Es-1*, *Es-2*, *Es-3* and *Es-5*), supernatant isocitrate dehydrogenase (*Idh-1*), supernatant malic enzyme (*Me-1*) (*Mdh-1* of Henderson [1966], Selander, Hunt and Yang [1969], and others; and *Mod-1* of Shows, Chapman and Ruddle [1970]), and mitochondrial malate dehydrogenase (*Mdh-2*) (*Mor-1* of Shows, Chapman and Ruddle [1970]).

Techniques of tissue preparation, electrophoresis and protein staining are described by Selander and Yang (1969). Because of the expense of staining for IDH, ME and MDH, the numbers of mice typed for these enzymes were reduced for most areas represented by samples exceeding 30. Mean sample sizes per area were 60 for esterases and 28 for other enzymes.

The distribution of allozymic variants in the house mouse has been studied in natural populations in North America (Petras *et al.*, 1969; Ruddle *et al.*,
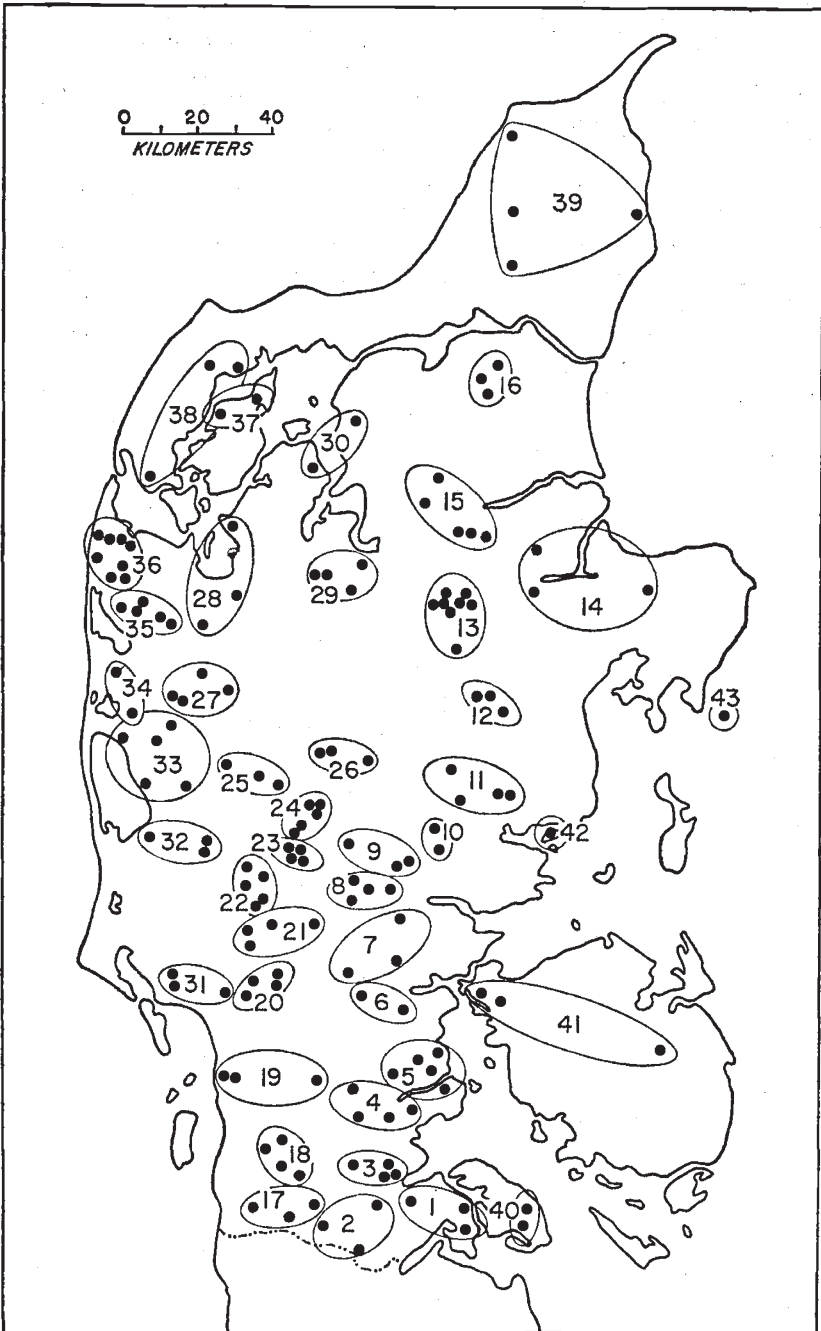
Fɪɢ. 3.—Sample localities for specimens of *Mus musculus*. Samples from individual farms (dots) are grouped into numbered sample areas, as indicated. The insular populations are as follows: 40, Als; 41, Fyn; 42, Alrø; and 43, Hjaelm. Falster Island (44) lies east of the area shown.

1969; Selander and Yang, 1969; Selander, Yang and Hunt, 1969), the Hawaiian Islands (Wheeler and Selander, 1972), parts of Britain (Berry and Murphy, 1970), and Jutland (Selander, Hunt and Yang, 1969). The distribution of alleles in inbred strains was summarised by Roderick *et al.* (1971) and Taylor (1972). The literature on breeding experiments demonstrating the generally codominant inheritance of allozymic variants is cited in these papers and in those mentioned in the following paragraph. A genetic analysis of variants in Jutland populations was presented by Wheeler (1972).

The linkage relationships of the loci considered are as follows: *Es-1*, *Es-2* and *Es-5* are in linkage group XVIII (Petras and Biddle, 1967; Popp, 1967; Ruddle, Shows and Roderick, 1969; Wheeler and Selander, 1972). Average recombination frequencies from several studies are 0·100 for *Es-1* and *Es-2*, 0·080 for *Es-1* and *Es-5*, and 0·009 for *Es-2* and *Es-5*, suggesting the linear arrangement *Es-1*, *Es-5*, *Es-2*, with only *Es-5* and *Es-2* tightly linked. *Es-3* is in group VII (Roderick, Hutton and Ruddle, 1970); *Idh-1* is in group XIII (Hutton and Roderick, 1970; Chapman, Ruddle and Roderick, 1971); and *Me-1* is in group II (Shows, Chapman and Ruddle, 1970). The linkage group for *Mdh-2* has not been determined.

4. RESULTS

(i) *Polymorphic variation*

Alleles are of two types, " major " alleles in moderate or high frequency in one or both semispecies and " minor " alleles in low frequencies with generally localised distribution. Geographic variation in frequencies of major alleles is shown in figs. 4 to 7. (Genotypic and allelic frequencies for all samples are presented by Hunt [1970].)

(a) *Esterase-1*

Populations of *musculus* in northern Jutland are monomorphic for the *Es-1ᵃ* allele, and those of *domesticus* near the German border are monomorphic for *Es-1ᵇ* (fig. 4). Introgression is more or less symmetrical north to south but is more extensive in western than in eastern Jutland. The distance between the 0·10 and 0·90 isofrequency lines for the *Es-1ᵃ* allele is 100 km. in the west but only 30 km. in the east. On Als Island (sample area 40), *Es-1ᵃ* has a frequency of 0·04, presumably reflecting introgression from the adjacent mainland, but other insular populations (Fyn, 41; Alrø, 42; Hjaelm, 43) are monomorphic for *Es-1ᵇ*.

A minor null allele, *Es-1ᶜ*, occurs at a frequency of 0·18 in sample area 39 in northern Jutland.

(b) *Esterase-2*

Six alleles are represented, two of which, *Es-2ᵇ* and *Es-2ᶜ*, are major. Shown in fig. 4 are the adjusted (relative) frequencies of the major alleles, calculated by eliminating minor alleles. *Es-2ᵇ* and *Es-2ᶜ* are essentially fixed in *domesticus* and *musculus*, respectively. Introgression is asymmetrical north to south: *Es-2ᵇ* penetrates to the Limfjord, but there is little introgression of *Es-2ᶜ* into southern populations. Again, the zone of transition in allele frequencies is broader in the west than in the east; distances between the 0·10 and 0·90 isofrequency lines for *Es-2ᶜ* are 120 and 45 km. respectively.

Populations on Als and Fyn islands are slightly introgressed from the mainland.

$Es\text{-}2^a$, a null allele, was detected in homozygous state in five sample areas in north-central Jutland. This allele may also occur in low frequency elsewhere on the peninsula, but, because it is undetectable in heterozygous condition, we could not determine its frequency directly. Maximum likelihood methods of estimating frequencies of null alleles from heterozygote
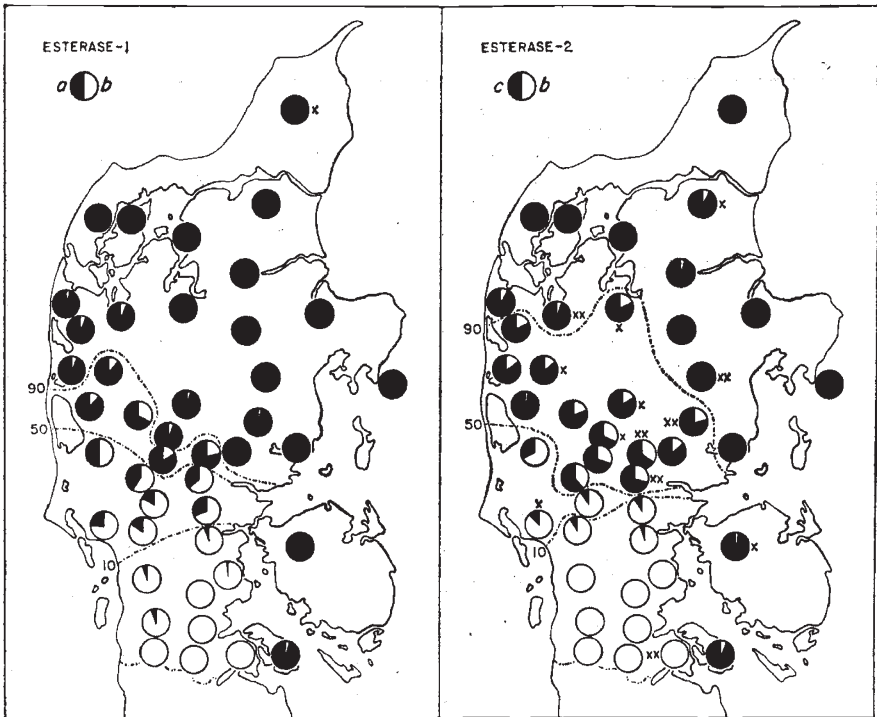

Fig. 4.—Geographic variation in frequency of major alleles at the $Es\text{-}1$ and $Es\text{-}2$ loci. Isofrequency lines for the $Es\text{-}1^a$ and $Es\text{-}2^c$ alleles are indicated. The occurrence of minor alleles is indicated by an X.

deficiencies in samples were considered inappropriate because of complications of the Wahlund effect (Selander, Yang and Hunt, 1969). $Es\text{-}2^e$ occurs in low frequency in eight sample areas, $Es\text{-}2^f$ in five, and $Es\text{-}2^g$ in two.

(c) *Esterase-3*

Populations in 43 of the 44 areas sampled are polymorphic for $Es\text{-}3^b$ and $Es\text{-}3^c$ (fig. 5). There is no conspicuous geographic variation in allele frequencies, but $Es\text{-}3^b$ occurs in slightly higher frequency, on the average, in the southern and western parts of the peninsula than in the north-eastern region occupied by relatively " pure " populations of *musculus*. This pattern is consistent with evidence from other loci of a more extensive introgression in the west than in the east. The frequency of $Es\text{-}3^b$ is surprisingly low (0·09) in sample area 1, being only slightly higher than that (0·06) on the adjacent Als Island.

A null allele, *Es-3^e*, was recorded in three sample areas, and *Es-3^f* was found in four areas.

(d) *Esterase-5*

Phenotypic variation in esterase-5 involves the presence or absence of a band of enzymatic activity. Presence results from a dominant allele, *Es-5^b*, while absence reflects homozygosity of the null *Es-5^a* allele (Petras and Biddle, 1967). *Es-5^a* is fixed or nearly so in *musculus*, but *domesticus* is polymorphic, with *Es-5^b* in frequencies of approximately 0·20 to 0·30. In fig. 5, variation at the *Es-5* locus is expressed in terms of the frequency of the homozygous
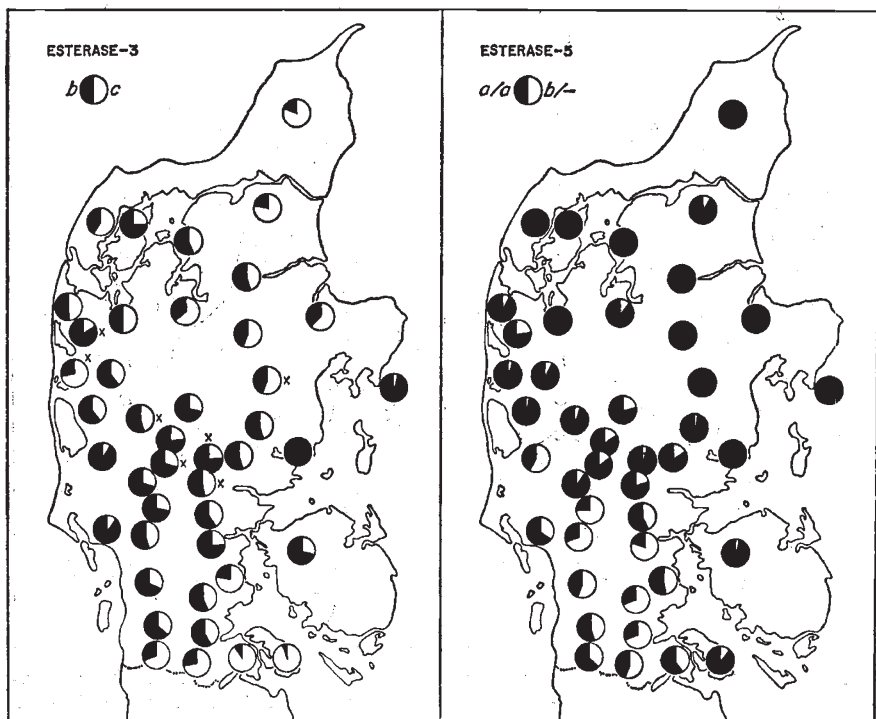

Fig. 5.—Geographic variation in frequency of major alleles at the *Es-3* locus and of genotypes at the *Es-5* locus. The occurrence of minor *Es-3* alleles is indicated by an X.

Es-5^a phenotype. The introgressive pattern at the *Es-5* locus is similar to that at *Es-2*. *Es-5^b* penetrates far into *musculus*, particularly in the west, but the relative uniformity of frequencies in southern populations indicates that little introgression is occurring from *musculus* to *domesticus*. Populations on Als and Fyn islands apparently are slightly introgressed from the mainland.

(e) *Isocitrate dehydrogenase-1*

Idh-1^a and *Idh-1^b* are, with few exceptions, alternately fixed in populations of *domesticus* and *musculus*, respectively (fig. 6). Introgression is symmetrical north to south, as in the case of *Es-1*, but asymmetrical east to west. If the presence of *Idh-1^b* in southern Jutland is in fact the result of introgression, this is the only locus at which a *musculus* allele has penetrated all the way to the German border. Introgression of *Idh-1^a* from *domesticus* also appears

unusually extensive, with this allele being represented in areas beyond the Limfjord (38 and 39). The presence of *Idh-1^b* at a frequency of 0·12 in sample area 1 suggests gene flow from Als Island, where populations appear also to be introgressed from the mainland. *Idh-1^b* is fixed in other insular populations.
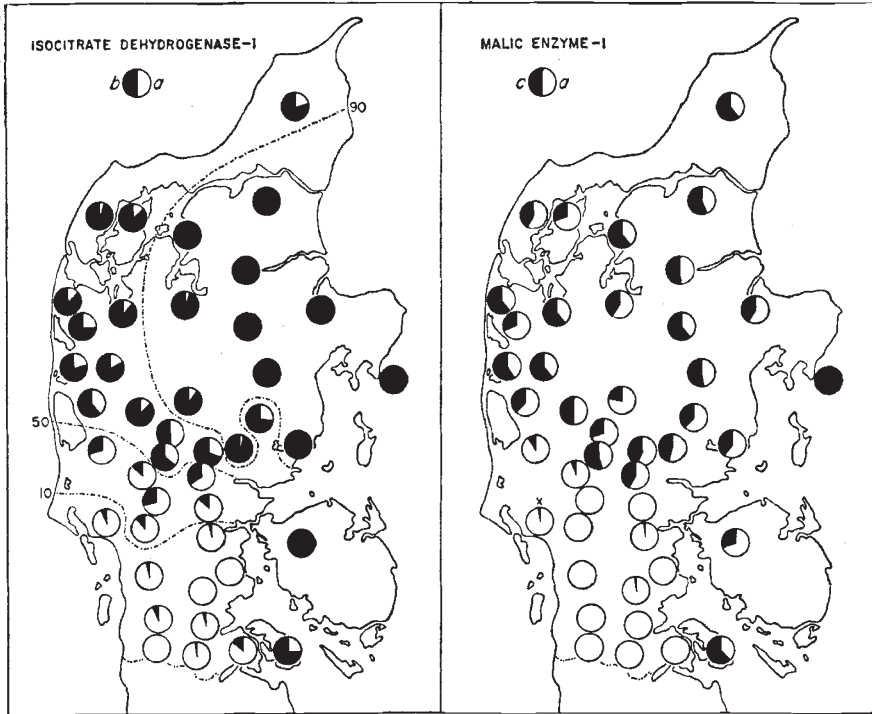

FIG. 6.—Geographic variation in frequency of major alleles at the *Idh-1* and *Me-1* loci. The occurrence of the minor allele *Me-1^d* is indicated by an X.

(f) *Malic enzyme-1*

Domesticus is monomorphic for *Me-1^a*, while *musculus* is polymorphic for *Me-1^a* and *Me-1^c* (fig. 6). The transition in central Jutland is very sharp, and there is little evidence of introgression of *Me-1^a* into northern populations, even on the western side of the peninsula. Some penetration of *Me-1^c* into southern populations is apparent, however.

A third allele, *Me-1^d*, was found in sample area 31 in south-western Jutland.

(g) *Malate dehydrogenase-2*

The pattern of variation at *Mdh-2* is similar to that at *Me-1* in that *domesticus* is monomorphic (*Mdh-2^b*) and *musculus* is polymorphic for two alleles (*Mdh-2^b* and *Mdh-2^c*). Introgression from *musculus* into *domesticus* is not apparent, but the relatively high frequency of *Mdh-2^b* in north-western Jutland presumably reflects extensive introgression from the south (fig. 7).

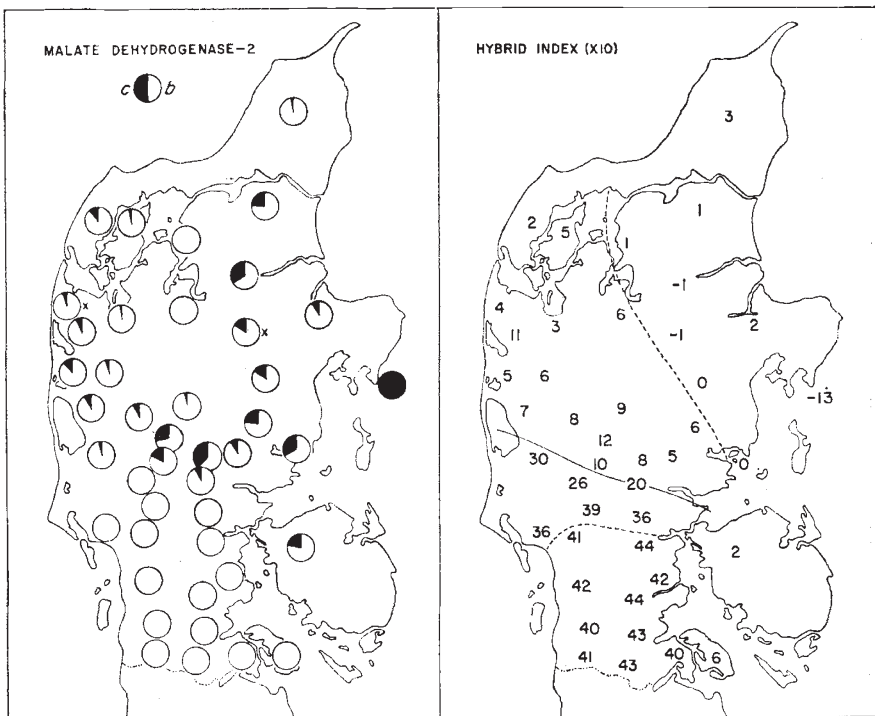A third allele, *Mdh-2^a*, was found in two sample areas in northern Jutland.

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Fig. 7.—Left: Geographic variation in frequency of major alleles at the *Mdh-2* locus. The occurrence of the minor allele *Mdh-2ᵃ* is indicated by an X. Right: Geographic variation in hybrid index score (*I* × 10) based on six polymorphic loci. Dashed lines indicate limits of introgression, as reflected by *I*, and the continuous line indicates the centre of the zone of hybridisation.

(ii) *Hybrid index*

To measure overall genetic character, a hybrid index score (*I*) for each sample area was computed as follows:

$$I = f_{Es-1b} + f_{Es-2b} + f_{Idh-1a} + (1 - f_{Es-5a/Es-5a}) + (f_{Me-1a} - 0.487)$$
$$+ (f_{Mdh-2b} - 0.818).$$

Frequencies of alleles are adjusted ones, and $f_{Es-5a/Es-5a}$ is the frequency of the null phenotype. The value 0·487 is the mean frequency of *Me-1ᵃ* in sample areas 12 through 15, representing minimally introgressed *musculus*, and 0·818 is the mean frequency of the *Mdh-2ᵇ* allele in these areas. The index is designed to yield highest scores (∼4·2) for " pure " populations of *domesticus* (2, 3, 17 and 18) and lowest scores (∼0·0) for those of *musculus* (12 to 15). *Es-3* was excluded because it provides little information on introgression.

The centre of the zone of hybridisation can be represented as the 2·1 isofrequency line, which lies between sample areas 7 and 8 in eastern Jutland, between 22 and 23 in the centre of the peninsula, and between 32 and 33 in the west (fig. 7). North-south and east-west introgressive asymmetries are readily apparent.

To illustrate the transition from *musculus* to *domesticus* in greater resolution, we have in fig. 8 shown hybrid index values for individual farms in central Jutland for which relatively large samples are available. Most of the transition in the eastern region occurs over a distance of less than 20 km. Interfarm heterogeneity within sample areas also is demonstrated in fig. 8.
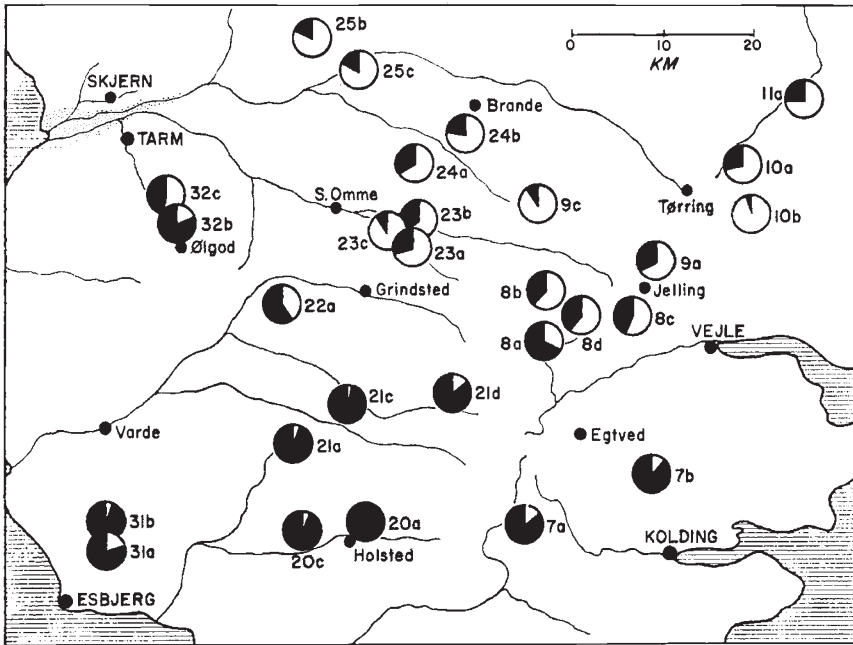

Fig. 8.—Interfarm variation in overall genic character at six polymorphic loci in central Jutland. Each circle represents a farm. Black areas of circles are proportional to hybrid index values, expressed as percentages of 4·2, the average value for samples of " pure " *domesticus*. Marshland between Skjern and Tarm is indicated by stippling.

(iii) *Degree of interbreeding*

Evidence from the field and laboratory indicates that, if interbreeding between *musculus* and *domesticus* is not entirely free, at least there is no strong tendency for assortative mating. In samples from individual farms within the zone we have not detected evidence of selective mating, the proportions of genotypes in samples from individual farms being similar to those expected in panmictic populations (data in Hunt, 1970; see also table 2).

5. DISCUSSION

(i) *Comparison with Ursin's earlier analysis*

The centre of the zone of hybridisation defined by Ursin's (1952) study of pelage colour and skin and skeletal dimensions extended from Fredericia (near Vejle) west through Grindsted to a point between Tarm and Skjern (see localities in fig. 8). That this is also the line defined by our analysis indicates that the centre has not shifted in the 20 years since Ursin's study.

Because the tail and hindfoot were slightly shorter in *musculus* populations north of the Limfjord than in those south of this channel, Ursin concluded that " weak gene-flow " from *domesticus* could be traced north to the Limfjord,

an inference confirmed by our demonstration of introgression in the western part of the peninsula at least as far north as Mors Island (37) in the Limfjord (fig. 7). (In the case of *Idh-1*, introgression may extend to the northern tip of the peninsula.) Ursin's data suggested a north-south asymmetry, for he could not detect with certainty introgressive effects south of the Vejle-Grindsted-Skjern line. We have shown that introgression is relatively limited south of the centre of the zone (fig. 7), especially in the east, although at *Es-1* and *Idh-1* it can be traced almost to the German border. The east-west asymmetry apparent from our study was also suggested by Ursin's data.

Populations on islands east of the Jutland Peninsula were assigned to *musculus* by Ursin, with the comment that they are similar to those north of the Limfjord. Our sample from Falster, a large island 120 km. east of the peninsula, is " pure " *musculus* ($I = -0.53$). Similarly, the Fyn population is not appreciably introgressed ($I = 0.22$), but the Als population ($I = 0.55$) shows slight introgression at most loci. The clearest indication of gene flow from Als to the adjacent mainland is provided by the relatively high frequency of *Idh-1^b* in sample area 1 (fig. 6).

Because the narrow channels separating Fyn and Als from the mainland are bridged by highways along which there is a heavy traffic of agricultural products in which mice could be carried, the severe restriction of gene flow is surprising. It may be significant that in climate and soil type Fyn and Als resemble the north-eastern area of Jutland occupied by *musculus* rather than the adjacent mainland inhabited by *domesticus* (see fig. 2).

Genic heterozygosity levels are " normal " on the large islands of Falster, Fyn, and Als. On the small island of Alrø, *Es-3*, a locus polymorphic in all mainland populations, is monomorphic (fig. 5). Hjaelm Island is occupied by a small population presumably founded by a few individuals and dependent for its existence on the lighthouse station located there. *Me-1* and *Mdh-2*, which are polymorphic in *musculus* on the adjacent mainland, have been fixed on Hjaelm (figs. 6 and 7), presumably through the founder effect or genetic drift, but *Es-3* remains weakly polymorphic (fig. 5).

(ii) *Position and width of the zone*

(a) *Position*

The question of whether hybridisation between *musculus* and *domesticus* is stable or transient pertains to both the location and width of the zone. As noted earlier, circumstantial evidence of an eastward shift in the zone from its original position is provided by its present disjunction in Jutland and Germany. The fact that these zones lie in regions of transition between Atlantic and continental climates suggests that climatic factors influence their positions (see also beyond). Since *musculus* and *domesticus* are almost entirely commensal in habit, their distributions and densities also undoubtedly have been influenced by historical changes in agricultural practices and the distribution of human populations. If, as suggested by Ursin (1952), the two forms meet where they are equally well adapted to ecological conditions, temporal variation in these conditions will preclude long-term stability of position of the zones.

How long *musculus* and *domesticus* have been in contact along their present front in central Jutland is unknown, but the western part of the zone cannot have existed in its present form prior to the mid-nineteenth century. Through prehistoric and early Christian times, much of Jutland was covered with oak

and beech forest (Davies, 1944). This habitat and the scattered farming communities would have supported populations of *Mus*, but the non-forested sandy outwash plains and marshy areas in the west probably would have been only sparsely and locally inhabited. In eastern Jutland the zone could have existed in much its present form and position for centuries, following the extensive destruction of forests in medieval times, but only tenuous avenues of contact between *musculus* and *domesticus* could have existed in the west prior to the 1850s, when a major programme of reclamation of heathlands and improvement of soil conditions was initiated, permitting an extensive development of agriculture on the sandhill islands and plains. Similarly, prior to the programme of reclamation of marshlands initiated in south-western Jutland in the mid-nineteenth century, human settlement was concentrated on scattered islands of drier land rising from meadows and bogs. The extensive peat bogs of northern Jutland and marshlands adjacent to the Limfjord, now partly reclaimed, also may have affected the distribution of *musculus* in earlier times.

Ursin emphasised the abruptness of the transition between *musculus* and *domesticus*, noting (1952, p. 235) that " populations with conspicuous hybrid characters apparently are met with only within an area the breadth of which is about 50 km. . . . Only within a zone of at most a few kilometres are the hybrid characters so pronounced that it is doubtful to what subspecies the population is most nearly related." The transition in overall genetic character in central Jutland is shown in fig. 9, in which hybrid index scores for sample areas along three transects are plotted against distance from south to north on the peninsula. Along the Vejle transect in the east (sample areas 1 to 16), " pure " *musculus* and " pure " *domesticus* populations are found within 80 km. of one another, and 90 per cent. of the transition in hybrid index occurs over a distance of 20 km. But along the Skjern transect in the west introgression is evident for a distance of at least 50 km. south of the centre of the zone and may extend northward up the coast beyond the Limfjord, a distance of more than 200 km.

Minor variation among loci in position of the mid-line of the zone probably is unimportant, considering the large part played by stochastic processes in determining allele frequencies at individual farms. Recall that we are dealing with disjunct, semi-isolated populations on farms, and that marked genetic heterogeneity exists among farms within small regions, largely as a result of founder effects (Selander, 1970). Founder effects and sampling error are probably also responsible for the few obvious " reversals " in the gradient of character transition detected in our study. As shown in fig. 7, sample area 23 has a lower hybrid index than area 24, owing to an unexpectedly high frequency of the *musculus* alleles $Idh\text{-}1^b$ and $Me\text{-}1^c$ (fig. 6). (Allele frequencies at other loci in area 23 are " properly " intermediate between those of areas 22 and 24.) Another reversal occurs in the west, where the hybrid index for sample area 35 is unexpectedly high (1·1) as a consequence of variation at *Es-2*, *Es-5*, *Idh-1* and *Me-1*.

The centre of the zone does not correspond to any sharp natural or man-made ecological discontinuity or to any obvious barrier to dispersal; and at least in the central and western parts of the peninsula it does not correspond to a steepening of gradients of environmental factors. The centre in the east lies near Vejle, where total yearly precipitation decreases northward from 800 to 625 mm. over a distance of 30 km. (figs. 2 and 9). Elevation is also
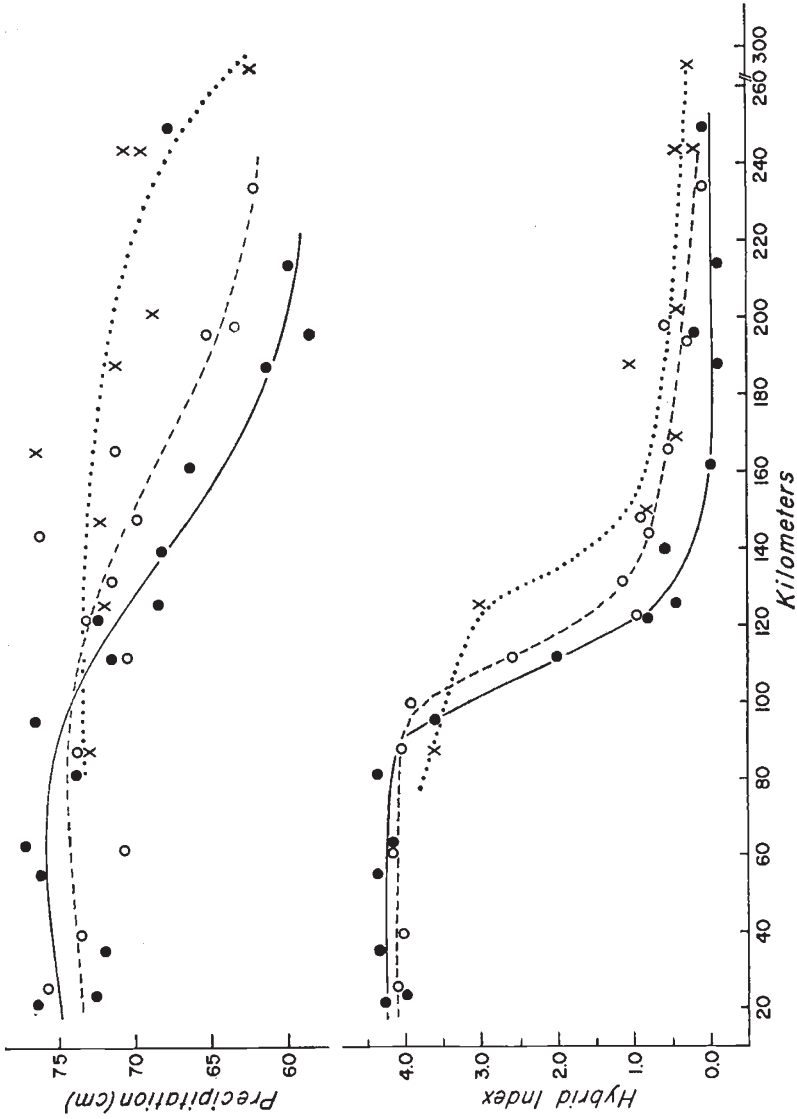
Fig. 9.—Variation in total yearly precipitation (above) and in hybrid index value (below) along three south-to-north transects through the Jutland Peninsula. Dots and solid line, Vejle transect (sample areas 1-16); circles and dashed line, Grindsted transect (17-30); Xs and dotted line, Skjern transect (31-39). Distance is measured in kilometres from the German border.

increasing in this region, so that areas above 100 m. occur just north of the mid-line. In the west, however, environmental correlates with the centre of the zone are less apparent. If precipitation alone were determining the position of the zone, we might expect the mid-line to lie near the south-western border of the Limfjord. Rather, it falls between Skjern and Tarm, where there is no abrupt change in precipitation, elevation, or soil type.

As shown in fig. 8, it is not apparent that the streams in Jutland affect gene flow in the central and eastern parts of the peninsula. For example, the characters of populations north (23b) and south (23a) of the Grindsted River are similar, as previously noted by Ursin (1952). However, the extensive marshes between Skjern and Tarm, where the Skjern and Grindsted rivers flow into the Ringkøbing Fjord, may impede dispersal. Ursin identified *musculus* at Skjern and *domesticus* at Tarm, only about 5 km. distant. Our material from near Tarm is strongly *domesticus*-like in character ($I = 3 \cdot 0$), while that from just north of Skjern is strongly *musculus*-like ($I = 0 \cdot 7$). However, a similar change in I occurs in an equally short distance (~ 20 km.) in the eastern part of the zone, where there are no obvious barriers to migration. In the east, the partial barrier to dispersal formed by the Vejle Fjord and the forested hills between Vejle and Jelling does not seem sufficient to account for the abruptness of the transition. Surprisingly, patterns of variation do not appear to be influenced by the Limfjord, which at least under more primitive conditions would have been a major barrier to dispersal of mice.

(b) *Width*

If the width of the hybrid zone in Jutland is not stable, the rate of expansion or contraction must be extremely low, for it is likely that *musculus* and *domesticus* have been hybridising in cental Europe for thousands of years. For *Mus* at least, we cannot agree with Remington's (1968, p. 375) suggestion that " the amount of natural hybridizing is commonly more a measure of recency of first sympatry than a measure of the amount of genetic dissimilarity . . .". At the same time, we cannot judge whether the width of the zone is slowly changing. Bigelow's (1965) suggestion that in transient zones trends toward " incorporation " should be detectable within a few generations may be an overly optimistic assessment of such situations. Computer simulations of zones of secondary contact or of clines of variation maintained by the opposing forces of selection and gene flow (Jain and Bradshaw, 1966; Crosby, 1969; Cook, 1972) emphasise the slowness with which equilibrium conditions are attained. With weak selection against introgressant alleles and a low migration rate, several thousand generations may be required to reach equilibrium at a locus initially fixed for different alleles in the parental forms. As noted by Fincham (1972) in regard to balanced polymorphisms, " most land environments may not be sufficiently stable for long enough periods for true equilibria ever to be attained ". In any event, the question of absolute or relative stability is in the present case of little consequence in attempting to understand factors determining the extent of introgression at different loci.

A notable feature of the zone is that introgression at most loci is more extensive into *musculus* than into *domesticus*. This type of asymmetry is a common feature of allopatric hybrid zones (Short, 1965; Yang and Selander, 1968). In the case of *Mus* it could reflect one or a combination of conditions,

including (1) greater interfarm migration in *musculus* than in *domesticus*, or a net northward dispersal; and (2) lesser intensity of selection against introgressant alleles in *musculus* populations than in those of *domesticus*.

Variation in width is also a common feature of allopatric hybrid zones, as early demonstrated by Meise (1928) for *Corvus* in Europe, and in some cases such variation is clearly associated with habitat features (Yang and Selander, 1968). The east-west asymmetry in width of the *Mus* zone corresponds to variation in steepness of environmental gradients, particularly precipitation (fig. 9), but also to elevation and, to some degree, soil type. Introgression of *domesticus* into *musculus* is relatively extensive in the west, where climatic conditions similar to those of southern Jutland extend up the coast even to the north-western part of the peninsula beyond the Limfjord. This feature of variation argues that selection by environmental factors has some part in limiting introgression.

Theoretically, the observed east-west asymmetry in width could be caused by regional differences in rate of migration. For example, a greater density of farms supporting mouse populations might facilitate interfarm migration, thus producing a broader transition in genetic character. But we have no reason to suspect that migration rate is in fact higher in the west than in the east.

The lesser extent of introgression where extensive contact and hybridisation presumably have existed for the longest period is not the result of development of incipient reproductive isolating " mechanisms " (see theory of Dobzhansky, 1940, 1970), since, as noted earlier, we have no evidence of non-random mating or of sterility barriers in any part of the zone. It is also unlikely that earlier chance establishment of parental populations north and south of the present centre of the zone is responsible for the asymmetry, since the zone is narrowest where there has been the longest period of time available for colonisation.

In an attempt to identify particular environmental variables regulating introgression, we performed a step-wise multiple linear regression of hybrid index values for 17 sample areas near the mid-line of the zone against the following independent variables: altitude, total yearly precipitation, July precipitation, February precipitation, mean yearly temperature, mean July temperature, and mean February temperature (data from Danske Meteorol. Inst., 1933, representing averages for the period 1886-1925). The fraction (R^2) of the sum of squares of I attributable to the regression is only 0·50, of which almost half (0·23) is associated with July precipitation. Repeating the analysis with latitude as one of the independent variables, we obtained an R^2 of 0·88, of which 0·77 results from the effect of latitude. Hence, considered apart from latitude, with which they are strongly correlated, precipitation, temperature, and altitude are not very useful " predictors " of the overall genetic character of populations in the hybrid zone. Either we have not identified all the important environmental variables or there are other factors influencing patterns of introgression.

(iii) *Differential introgression and selection*

If selection is a factor controlling the extent of introgression, it is, as Mayr (1963) has suggested, unlikely that it will affect all loci with the same intensity. Rather, it is to be expected that the progress of some alien alleles will

be checked near the centre of the hybrid zone, while the flow of others will proceed varying distances into regions occupied by the parental populations.

Our analysis suggests that the geographic pattern of gene flow is distinctive for each locus. Variation is especially apparent among the three loci (*Es-1, Es-2* and *Idh-1*) at which alternate alleles are fixed or nearly so in the two semispecies. *Idh-1ᵃ* has penetrated into *musculus* populations beyond the Limfjord, whereas *Es-1ᵇ* can be detected only as far north as the southwestern edge of the Limfjord. Introgression of *Mdh-2ᵇ* to regions north of the Limfjord is also suggested by the pattern of allele frequencies shown in fig. 7.

Introgression of *musculus* alleles into *domesticus* is relatively limited but similarly variable. *Idh-1ᵇ* reaches the German border, and *Es-1ᵃ* goes further south than *Es-2ᶜ*. There is a slight south-western introgression of *Es-5ᵃ*, but no apparent introgression of *Mdh-2ᶜ*.

Although linkage disequilibrium is difficult to assess in *Mus* populations because of their subdivided structure and the occurrence of null alleles at several of the loci, a comparison of allele frequencies at the linked loci *Es-1*,

TABLE 1

*Allele frequency correlations in central Jutland**

	Es-1ᵇ	Es-2ᵇ	Es-3ᵇ	Es-5ᵃ/ᵃ	Idh-1ᵃ	Me-1ᵃ	Mdh-2ᵇ
Es-1ᵇ	1·00	0·86	0·39	−0·70	0·88	0·73	0·36
Es-2ᵇ		1·00	0·51	−0·83	0·84	0·84	0·25
Es-3ᵇ			1·00	−0·51	0·52	0·60	−0·08
Es-5ᵃ/ᵃ				1·00	−0·61	−0·76	−0·39
Idh-1ᵃ					1·00	0·81	0·21
Me-1ᵃ						1·00	0·32
Mdh-2ᵇ							1·00

* 20 sample areas (7-11, 21-29, and 31-36).

Es-2 and *Es-5* suggests that linkage between the loci studied is not an important factor affecting patterns of introgression. Shown in table 1 are correlations of allele frequencies between pairs of loci in the primary area in which introgression is occurring (bounded by dashed lines in fig. 7). There are strong correlations for combinations of *Es-1, Es-2* and *Es-5*, but correlation is equally strong for the unlinked pairs *Es-1ᵇ—Idh-1ᵃ*, *Es-2ᵇ—Idh-1ᵃ* and *Es-2ᵇ—Me-1ᵃ*. Near the centre of the hybrid zone individual farm populations are in equilibrium with regard to *Es-1* and *Es-5*, and this is generally so also for *Es-1* and *Es-2* (table 2). But mild disequilibrium between the closely linked *Es-2* and *Es-5* loci is seen at about half the farms.

In the absence of relevant experimental data, our discussion of how selection operates in the hybrid zone is necessarily speculative. Following Ursin (1952), we conclude that the zone lies in a region where gradients of climatic factors, particularly precipitation, create environments to which the semispecies are equally well adapted. The east-west asymmetry in width of the zone reflects variation in extent of the area in which the critical set of intermediate environmental conditions is distributed. Direct selective pressures of the external environment may influence some aspects of allele frequency variation, but it is likely that selection resulting from the disruption of the respective co-adapted gene complexes of the parental forms is also involved. Particularly pertinent to the argument that the selective value of alleles at individual loci is determined in part by the internal genetic environment are

the extreme steepness of the gradient of transition in genetic character and the fact that frequencies at all loci have a major shift along the same mid-point line. If the loci were simply responding to external environmental gradients, the mid-points of frequency would vary in position, although sigmoidal curves would result from the interaction of migration and selective gradients (Haldane, 1948; Fisher, 1950). Apparently selective coefficients for all alleles change sign along the Vejle-Grindsted-Skjern line. Our failure to find close relationships between geographic patterns of external environmental variables and introgressive patterns at individual loci also points to

TABLE 2

Genotypic combinations at paired loci in linkage group XVIII at farms in central Jutland

Genotypic combination	Farm: Observed (expected)					
	22a	23a	25c	27b	32b	32c
Es-1/Es-2						
aa/bb	1 (2)	1 (1)	0 (0)	0 (0)	8 (7)	2 (2)
aa/cc	3 (3)	11 (11)	4 (7)	14 (14)	0 (0)	20 (16)
aa/bc	3 (2)	17 (18)	11 (8)	3 (3)	0 (1)	0 (4)
bb/bb	8 (5)	0 (0)	0 (0)	0 (0)	26 (25)	0 (0)
bb/cc	7 (8)	0 (0)	3 (2)	0 (0)	0 (0)	0 (0)
bb/bc	2 (5)	1 (1)	2 (3)	0 (0)	2 (3)	0 (0)
ab/bb	1 (3)	0 (0)	0 (0)	0 (0)	25 (27)	2 (2)
ab/cc	6 (5)	3 (3)	11 (9)	6 (6)	0 (0)	6 (10)
ab/bc	5 (3)	5 (5)	8 (10)	2 (2)	5 (3)	6 (2)
$\chi^2_{(4)}$	7·54	0·94	3·67	0·19	2·41	12·54*
Es-1/Es-5						
aa/aa	6 (6)	21 (23)	14 (14)	16 (15)	4 (2)	17 (15)
aa/b-	1 (1)	8 (6)	1 (1)	1 (2)	4 (6)	5 (7)
bb/aa	16 (16)	1 (1)	5 (5)	0 (0)	5 (8)	0 (0)
bb/b-	1 (1)	0 (0)	0 (0)	0 (0)	23 (20)	0 (0)
ab/aa	11 (11)	8 (6)	18 (18)	6 (7)	9 (8)	7 (9)
ab/b-	1 (1)	0 (2)	1 (1)	2 (1)	21 (22)	7 (5)
$\chi^2_{(3)}$	0·42	3·16	0·37	1·71	3·39	2·78
Es-2/Es-5						
bb/aa	9 (9)	1 (1)	0 (0)	0 (0)	12 (16)	1 (3)
bb/b-	1 (1)	0 (0)	0 (0)	0 (0)	47 (43)	3 (1)
cc/aa	14 (15)	14 (11)	18 (17)	20 (18)	0 (0)	20 (17)
cc/b-	2 (1)	1 (3)	0 (1)	0 (2)	0 (0)	6 (9)
bc/aa	10 (9)	15 (18)	19 (20)	2 (4)	6 (2)	3 (4)
bc/b-	0 (1)	8 (5)	2 (1)	3 (1)	1 (5)	3 (2)
$\chi^2_{(3)}$	1·33	6·73*	1·73	13·64**	13·58**	5·30

the genetic environment as a factor determining the distribution of alleles. We are thus suggesting that recombination products of the parental *musculus* and *domesticus* genotypes are selected against by a combination of external and internal environmental factors. A similar argument was advanced by Clarke (1968) and Clarke and Murray (1971) to explain certain aspects of the distribution of colour-morph frequencies in *Partula* and *Cepaea* snails. And Carson (1972) has emphasised the fact that one-to-one correlations between genes and external environmental parameters are unlikely because " the object of selection is an interacting system rather than a single gene ".

Disruption of the coadapted structure of the *musculus* gene pool may be causally related to the relatively widespread occurrence of minor *Es-2* and

Es-3 alleles in the northern part of the hybrid zone (figs. 4 and 5). Introgression from *domesticus* could modify the *musculus* pool in such a way that selective barriers to incorporation of new alleles are relaxed, or the minor alleles could even be favoured in low frequency in the " new " genetic environment created by introgression (see Stebbins, 1971).

Despite a long period of interbreeding not only in Jutland but also in Germany, the *musculus* and *domesticus* gene pools have not fused. Hence, our studies support the view that genetic isolation cannot be directly equated with reproductive isolation, since strongly integrated and co-adapted gene pools may be effectively protected against introgression even in the absence of reproductive isolation (Stebbins, 1950; Bigelow, 1965; Hagen and McPhail, 1970). Comparable cases have been analysed by Hagen (1967) in fishes and by Hall and Selander (1973) in lizards.

The behaviour of alleles at the structural loci examined in the present study does not seem readily compatible with the hypothesis that allozymic variants in populations and in closely allied species are selectively neutral (King and Jukes, 1969; Kimura and Ohta, 1971). However, the confirmed " neutralist " can point to the possibility that selection is affecting the chromosomal segments that the loci mark rather than the loci themselves. At present we see no way of resolving the problem of whether selection acts on the loci studied or on others with which they are very tightly linked. In any event, our study suggests that selection against potentially introgressive genes, presumably involving reduced fitness in backcross generations, is very strong, and that the degree of disruption of the coadapted parental gene complexes caused by introgression is not equal over all loci.

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