Genet. Res., Camb. (1961), **2**, *pp.* 249–271
With 5 text figures
Printed in Great Britain

On the distribution of recessive embryonic lethals in a natural population of *Coelopa frigida* (Fab.)

By B. BURNET*

Department of Zoology, King's College, Newcastle upon Tyne, 1

(*Received* 20 *December* 1960)

INTRODUCTION

There is now an extensive literature on the frequency of recessive lethals in natural populations but it is confined almost exclusively to a few species of *Drosophila*. Dubinin (1946) and Spencer (1947) have reviewed the earlier work, whilst more recent studies have been made by Pavan *et al.* (1951), Dobzhansky *et al.* (1955), Goldschmidt *et al.* (1955) and Paik (1960). Comparative studies show that the gross frequency of lethals tends to vary not only between species, but between different populations of the same species in different geographical areas. These differences have been interpreted as the reflection of differences in the size and breeding structure of populations in different habitats.

In view of the comparative lack of information on the frequency of lethals in natural populations other than of *Drosophila* it seems desirable to examine the situation in a different genus. The work to be described concerns such an investigation on a natural population of the littoral acalypterate fly *Coelopa frigida* (Fab.), which is widely distributed along the coasts of Northern Europe, forming well-defined populations breeding in beds of rotting seaweed cast up at high-water mark.

Drosophila workers generally infer the presence of a lethal in their material from the behaviour of balanced lethal marker stocks, and are able to map the position of a lethal factor without necessarily obtaining any information about its morphological effect. Consequently, the frequency in natural populations of *Drosophila* species of lethals acting at particular stages in the life-history has not received much attention. The females of many *Drosophila* species scatter their eggs at intervals so that the progeny of a female are not all at the same stage of development at any one time, but in the case of *Coelopa frigida* the eggs are layed in a clutch from which the larvae hatch together and are comparable at any point in time. This makes it easier to locate the effect of a lethal in the egg, larval or pupal stages. Advantage has been taken of this feature of *C. frigida* to collect lethals which exert their effect during a well-defined period of the life-history, in this case during embryonic development.

The colony on which the work was carried out consists of a small semi-isolated population in which all stages of the life-history occur together, confined within

* Now at the Agricultural Research Council's Poultry Research Centre, West Mains Road, Edinburgh, 9.

the area of a single wrackbed occupying an area rather less than 30 square metres. In contrast to previous investigations, samples taken from the population did not consist of mobile adult insects caught in traps but of larvae taken directly from their feeding sites *in situ* within the wrackbed. During two exploratory investigations it became clear that interpretation of the genetic content of such samples necessitated detailed information concerning the spatial distribution of the larvae to make possible an unbiased estimate of lethal gene frequencies. A brief account of features of the ecological structure of the population relevant to the estimation of gene frequencies is given at the beginning of the paper, and in the last section an attempt is made to integrate the genetics and ecology in order to draw some conclusions regarding the breeding structure of the population.

MATERIALS AND METHODS

A description of the morphology and taxonomy of *Coelopa frigida* (Fab.) and *Coelopa pilipes* (Hal.) is given by Burnet (1960). Descriptions of the larval stages are given by Egglishaw (1960) and Burnet (1960), and a detailed account of laboratory culture methods for *C. frigida* is given by Burnet & Thompson (1960).

The material was obtained from a small wrackbed at the foot of cliffs on the north side of the causeway connecting Currie's Point to St Mary's Island, Northumberland. Samples were taken from this colony for analysis annually from 1957 to 1959, and the adult flies hatched out in the laboratory. In the first analysis in 1957 small groups of about a dozen larvae each were collected at random on three separate days in October. In the second analysis in 1958 small groups of larvae were collected on one day in April. In the third analysis in 1959 the bed was divided into equal areas and single larvae collected at random from each so that the bed was sampled uniformly; this process was repeated on three separate days in March–April.

Cultivation

Single pair cultures are made up in half-pint milk bottles with a pad of cellulose wadding moistened with sea-water (3 g. wadding, 30 c.c. sea-water), and a piece of split *Laminaria* stipe about 5 cm. long. Each bottle is inspected for eggs after 12 hours. Females lay their eggs as a clutch with a mean of 76 eggs which overlap each other in rows and hatch within a few minutes of each other. Up to four clutches may be laid by a single female at intervals of one or two days. Both parents are removed from the culture bottle when the first clutch is laid and the quantity of *Laminaria* stipe made up to approximately 60 g. The life cycle takes 12 days at 25°C. Adult flies can be kept in fertile condition for several weeks on 3% solution of mannitol in a cold chamber at +5°C. Details of ecological and genetical methods are given in the appropriate sections.

ECOLOGICAL STRUCTURE OF THE POPULATION

Ecological Methods

An accurate estimate of the absolute numbers of individuals in the St Mary's colony is difficult to make. In the approach to this problem use was made of the

fact that the bed is normally destroyed by the monthly spring tides which reduce all but the adult population to zero. The census of adults was carried out at this minimum point. Flies released into the population were marked by means of Rotor Brilliant Red R, kindly supplied by the dyestuffs division of Imperial Chemical Industries. This marking powder gives an acetone spot with trace quantities which accumulate round the joints and genae. The method is described in detail by Macleod & Donnelly (1957).

The distribution and relative density of the larval population was studied by means of cores taken from the bed using a brass cylinder with a cutting edge fitted with handles and a plunger. A movable collar was fixed at 10 cm. from the cutting edge and cores measuring 5 cm. in diameter and 10 cm. in length taken from the bed. The free end of the core was cut away with scissors. The procedure for taking cores was to divide up the bed surface into half-metre quadrats and to take one core from each quadrat. Before each core was taken the dry surface layer was brushed aside to expose the compact humid layers beneath. The larvae, which are photophobic, are rarely found in this light surface layer. The cores in individual polythene bags were put into deep freeze at -5°C. to ensure that no larvae were able to pupate before being sorted. The larvae were separated from the wrack by washing and preserved in 50% alcohol for identification.

The habitat and wrack fauna

A description of the ecology of wrackbeds has been given by Backlund (1945), who worked on the coasts of Sweden and Finland, and Egglishaw (1960) has given a detailed account of the Coelopidae. The St Mary's colony consists of a typical wrackbed overlying boulders at the foot of low cliffs. The bed is normally destroyed and washed away by the monthly high-water spring tides but may occasionally remain intact for two months, depending on the stage of the weather. The bed consists mainly of the frond and stipe of *Laminaria* and *Fucus*, though other Phaeophyceae and Rhodophyceae may be included along with other marine rejectamenta. Pockets of heat rapidly develop within the bed, spreading as it settles down, and the temperature of the bed below a thin surface layer rises to 20–30°C. except where the wrack is loosely packed, dry, or mixed with sand.

The insect fauna consists mainly of adult Staphylinidae and Diptera and their larvae. *Thoracochaeta zosterae* (Sphaeroceridae) often occurs in large numbers, whilst *Fucellia maritima* (Muscidae) is present in small numbers during the summer. Of the five species of the family Coelopidae known to occur in Britain, only three occur at St Mary's. *Orygma luctuosa* breeds in the bed in spring and autumn, but the two principal inhabitants of the bed are *Coelopa frigida* and *C. pilipes*, which alternate in relative abundance according to the time of year. *C. frigida* is most abundant from September to February, but *C. pilipes* predominates during the summer months (Egglishaw, 1960). The change in relative abundance of the two species may be due to seasonal changes in the chemical composition of the *Phaeophyceae* described by Black (1948).

The size of the adult population

When a wrackbed is destroyed by the spring tides juvenile stages which have not completed development are washed away. The next bed formed from freshly deposited wrack is recolonized by a foundation population of adult flies from the old bed. The life-cycle, which lasts about 12 days at 25°C., allows development of the first generation which produces a second generation of flies which hatch before commencement of the next spring tide at the end of the 28-day cycle. A proportion of these adults forms the foundation population for the next population cycle. Because the females lay clutches over a period of days there is some overlap of the first and second generation and many second-generation juvenile progeny of late-hatching first-generation flies are unable to complete development before the wrackbed is destroyed. Thus the total number of individuals in the colony oscillates every alternate generation, and this oscillation is superimposed over the seasonal fluctuation in numbers. An estimate based on the mark-release-recapture method of the number of adult flies forming the foundation population in the new wrackbed for two successive cycles is shown in Table 1. The census for March 1959 shows the size of the population on which the genetic analysis for 1959 was conducted.

Table 1. *Estimates based on the mark-release-recapture method of the number of adult flies forming the foundation population in the St Mary's colony at the beginning of two successive population cycles at the end of the monthly spring tides*

	Release marked	Recaptures		Population size
		Marked	Unmarked	
February 1959	♀ 125	4	54	1813
	♂ 125	3	25	1166
March 1959	♀ 107	8	44	696
	♂ 105	5	27	672

The spatial dispersion of the larval population

For a species distributed at random within the habitat the number of individuals per random core or quadrat will be distributed according to the Poisson series with mean ($\bar{x}$) equal to the variance (s^2). The index of dispersion ($s^2/\bar{x}$) exceeds unity when the individuals tend to be aggregated or clumped in groups, and is less than unity when they are evenly distributed. An observed value for the index differs significantly from unity if the difference exceeds $2\sqrt{(2n/(n-1)^2)}$, where n is the number of cores examined. Table 2 shows the mean and standard deviation of the number of third instar larvae per core for *C. frigida*. The index of dispersion is many times greater than unity, indicating that the larvae are not randomly distributed but show evidence of aggregation or clumping.

Under these circumstances special methods are required for analysing the data. Evans (1953) has investigated the applicability of theoretical contagious distributions to data on plant and insect populations and has found that, in general,

Table 2. *Index of dispersion* ($s^2/\bar{x}$) *of third-instar larvae of* C. frigida. *Further explanation in text*

Date	$\bar{x}$	s	n	$s^2/\bar{x}$	$2\sqrt{(2n/(n-1))}$
Oct. 1958	77·7	83·2	90	91·2	0·30
Mar. 1959	1·92	13·1	80	89·5	0·32
Mar. 1959	8·75	19·4	75	43·2	0·33
Apr. 1959	28·3	37·4	65	49·7	0·36
Apr. 1959	7·31	18·4	58	46·2	0·38
June 1959	3·76	9·96	55	21·4	0·39
Nov. 1959	129	109	56	93·3	0·39

data from insect populations are best fitted by the negative binomial distribution. The proportionality between the mean and standard deviation in Table 2 is similar to that shown by Quenouille (1950) to require a transformation appropriate to the negative binomial distribution. Quenouille (1950) gives the transformation:

$$y = \frac{1}{\beta}\sinh^{-1}\beta\sqrt{(x+\tfrac{3}{8})} \qquad (1)$$

where β is the gradient of the standard deviation on the mean and x is the number of larvae per core. The addition of $\frac{3}{8}$ has been shown by Anscombe (1948) to achieve greater variance stability.

Factors affecting the spatial dispersion of the larvae

There are several factors which may influence the spatial dispersion of larvae. The most obvious possibilities are temperature, humidity, oxygen tension, or a tendency to form feeding aggregates. The first two are related in that only humid wrack heats up in the familiar manner. The warm layers of the bed are covered in a colloidal slime in which the larvae feed, often, except for the posterior spiracles, completely submerged in a semi-liquid stipe of the decomposing *Laminaria*. They are able to withstand wide variations in the osmotic pressure of the medium (Sutcliffe, 1960).

Temperature

During the larval census of June and November 1959 the temperature of the bed was taken at each position from which a core was taken. It is worth while to compare the relationship between density and temperature for third instar larvae of *Coelopa frigida* with that of *C. pilipes*, since these make an interesting study of two closely related species both occupying the same habitat. Fig. 1 shows the relationship between the mean number of larvae per core for each 1°C. interval for *C. frigida* and *C. pilipes* in June. The values on the ordinate correspond to the transformed scale of equation (1). Due to the low seasonal abundance of *C. frigida* at this time of year no larvae of this species were recorded at all in some temperature zones giving rise to a scatter of points. For *C. pilipes* the distribution consists of two parts. There is a rapid rise in density up to 21°C. followed by a plateau. In the November census shown in Fig. 2 the behaviour of the two species is reversed. There is a steady rise in density of *C. pilipes* over the whole temperature range, whereas with *C. frigida*, which at this time of year is the more abundant of

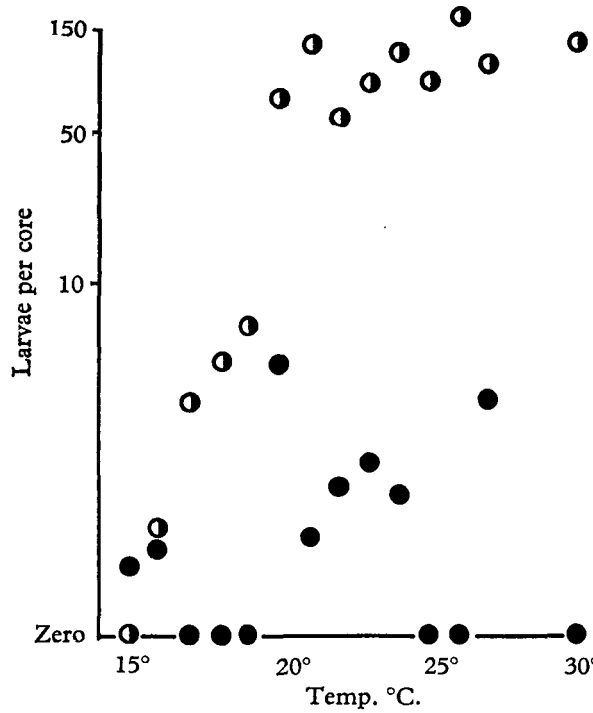

Fig. 1. Relation between mean larval density and temperature in the wrackbed in June. *C. frigida* solid circles, *C. pilipes* half solid circles.

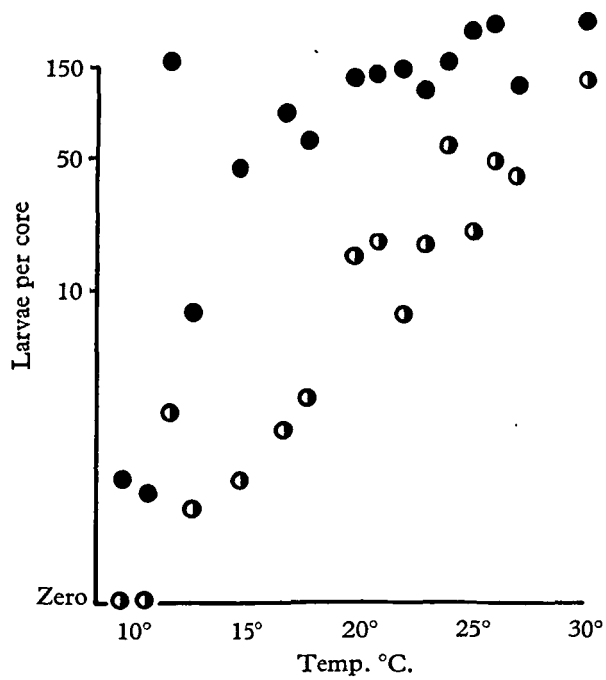

Fig. 2. Relation between mean larval density and temperature in the wrackbed in November. *C. frigida* solid circles, *C. pilipes* half solid circles.

https://doi.org/10.1017/S0016672300000732 Published online by Cambridge University Press

the two, there is a rapid rise in density followed by a plateau after 20°C. Comparison of Figs. 1 and 2 shows that for both species there is a positive correlation between larval density and temperature but that there is an upper limit to the density reached at about 20°C. by whichever species is seasonally abundant.

The increase of larval density with temperature can be visualized in two ways: either by larvae moving into high-temperature zones, or by adult flies exercising a preference for oviposition sites in zones of high temperature. The latter possibility was investigated in the following way. The apparatus consisted of two glass containers maintained at 15°C. and at 25°C. respectively, joined by a short polythene connecting cylinder. Oviposition sites of *Laminaria* frond were provided in each container. Twenty pairs of flies were introduced into the connecting cylinder and the number of clutches of eggs in each container scored after 48 hours under constant illumination. The results for three replicate experiments in Table 3 show that the flies exercised a marked preference for oviposition sites in the 25°C. container.

Table 3. *Number of clutches of eggs laid in three replicate experiments by twenty pairs of flies with a choice of oviposition sites at 15°C.* and *25°C. in mass culture*

Clutches laid	
15°C.	25°C.
3	23
0	17
2	18

Family aggregates

The mean larval density per core is positively correlated with temperature, but in the lower temperature intervals the mean tends to be derived from cores containing several larvae or none. This effect is not shown in the higher temperature range. A feature of these isolated cores is that the larvae within any particular core show a marked similarity in size. Since the females of *C. frigida* lay their eggs in clutches and the larvae hatch simultaneously this suggests that the larvae in these cores belong to the same family.

A convenient feature of the transformation expressed in equation (1) is that for a species distributed at random, the residual mean square has a value of 0·25. Individuals tend to be aggregated when the residual mean square is greater than 0·25 and to be evenly distributed when it is less than this value. Quenouille (1950) made use of this property to demonstrate the random distribution of ticks on sheep. Tables 4 and 5 show an analysis of variance of the transformed data from the larval census of June and November 1959. The mean square between temperatures has been removed from the total to give the residual mean square between cores which may be compared with the expected theoretical variance of 0·25 for random distribution, by means of an F test.

In Table 4 the results are shown for *C. frigida* in the June census. Over the range 15–30°C. the residual mean square is 0·4076, nearly twice the expected value

for random distribution of the larvae, suggesting that there are local aggregations of larvae. Fig. 2 showed that in the November census the relationship between mean larval density and temperature fell into two parts and these have been treated separately. In the range 10–20°C. the residual mean square is 0·5470, or more than twice the expected value for randomness. In the series 21–30°C., however, the residual mean square is 0·0927, very much lower than the expected value, suggesting that the larvae are more evenly distributed in this temperature range.

Table 4. *Analysis of the spatial dispersion of third-instar larvae of* Coelopa frigida

	June, 15–30°C.			November, 10–20°C.			November, 21–30°C.		
Source of variance	Degrees of freedom	Mean square	*F*	Degrees of freedom	Mean square	*F*	Degrees of freedom	Mean square	*F*
Total	54	0·440		27	1·266		27	0·102	
Between temps.	13	0·544	1·33	7	3·320	6·07*	7	0·129	1·40
Cores within temps.	41	0·408	1·63*	20	0·547	2·19*	20	0·092	2·71*
Theoretical variance		0·25			0·25			0·25	

* Significant at the 1% level.

Table 5 shows the results for *C. pilipes*. In the June census the residual mean square for the range 15–21°C. is 1·1046, over four times larger than the expectation for randomness. In the range 22–23°C., in contrast to the results obtained with *C. frigida*, the residual mean square does not differ significantly from the expected value for randomness. Similarly, the results for November are not significantly higher than the expected value.

Table 5. *Analysis of the spatial dispersion of third-instar larvae of* Coelopa pilipes

	June, 15–21°C.			June, 22–30°C.			November, 12–30°C.		
Source of variance	Degrees of freedom	Mean square	*F*	Degrees of freedom	Mean square	*F*	Degrees of freedom	Mean square	*F*
Total	32	1·887		20	0·257		55	0·865	
Between temps.	6	5·275	4·77*	6	0·063	0·19	15	2·335	7·45*
Cores within temps.	26	1·105	4·42*	14	0·339	1·32	40	0·313	1·25
Theoretical variance		0·25			0·25			0·25	

* Significant at the 1% level.

Statistical analysis shows that for *C. frigida* there is a real tendency for the formation of aggregations of larvae, which may be regarded as family aggregates with a limited radius of dispersal from the original oviposition site. Because of the preference of adult flies for oviposition sites in zones of high temperature within the bed, the increase in larval density with temperature can be visualized as due to

a decrease in the average distance between neighbouring aggregates due to increase in number of clutches per unit area. The even distribution of the larvae in the high-temperature range is due to the larvae approaching a saturation density or upper limit to the number of larvae which can be accommodated in unit volume of food material, similar to the effect demonstrated under laboratory conditions by Burnet & Thompson (1960). An important consideration lies in the effect temperature has on general activity, which may cause the radii of dispersal to be longer at the higher temperatures, so assisting any tendency of the aggregates to coalesce and produce an apparent uniformity in the distribution.

GENETIC STRUCTURE OF THE POPULATION

Isolation of lethals

Embryonic lethals are isolated by the F_2 sib-mating method. This consists of brother-sister mating of the progeny of a pair of wild flies. One-quarter of these sib-matings is expected to consist of parents both heterozygous for a lethal carried by one of the original wild pair, which will reveal its presence in the F_2 by the proportion of hatching to non-hatching eggs in their first clutch. Since the average number of eggs per clutch is 76, the probability of misclassifying a clutch is very small. A more important source of error lies in the actual number of sib-matings examined. If n sib-pairs are scored, the probability of missing a lethal is $(3/4)^n$, so that $1-(3/4)^n$ is the proportion of lethals in the original wild pairs that we expect to isolate. The analyses of 1957 and 1958 were exploratory and clutches from only four and six sib-pairs were examined on the average, but in the 1959 analysis, in which greater accuracy was required, clutches from eight pairs were usually examined, testing 90 % of the variability in each original pair of wild flies. The number of lethals isolated in each of the three years is shown in Table 6.

Table 6. *Gross frequency of embryonic lethals in three analyses of the St Mary's colony*

	Gametes tested	Lethals	Gross frequency	Average number of sib-matings examined
1957	192	19	0·0989	4
1958	104	6	0·0577	6
1959	232	19	0·0819	8

For convenience three morphological types of lethal embryo are distinguished, based on the classification of Hadorn (1951). These are: E stage in which there is the central yolk mass still visible; secondly E/L stage in which the lethal embryo is segmented and has a recognizable cephalopharyngeal apparatus. Within the E/L group two types are recognized: E/L.N in which the embryo shows no apparent morphological abnormality; and E/L.ABN in which morphological abnormalities are clearly recognizable in whole-mount preparations. These broad

morphological types are easily distinguished by dechorionating unhatched eggs in 5% solution of sodium hypochlorite.

Tests for allelism between lethals

Two techniques for testing allelism between lethals were investigated.

Method I. Two cultures each from a pair of flies giving a lethal segregation in the first clutch were taken from each of two lines to be tested and the larvae raised to maturity. As the adults hatched, the sexes were separated from each culture and reciprocal pair matings made between the two lines. Females normally hatch before males and will not copulate until 6–12 hours after eclosion so that virgin females can be separated without difficulty. The first clutch from each pair was inspected for a lethal segregation. If the two lines contain allelic lethals, $\frac{4}{9}$ of the pairs examined will give a 3:1 segregation in the first clutch.

This method proved to be cumbersome in practice, and was used only for cross tests of the lethals isolated in 1957.

Method II. Test crosses using wild-type flies and flies homozygous for three unlinked visible factors, white eye, sherry eye and cut wing, have shown that the last impregnation of the female is the effective one. However, *C. frigida* females in single pair cultures may require reimpregnation after the first clutch of eggs is laid, since the second and third clutches from females isolated after the first impregnation often contain unfertilized eggs only. This information provides a method of testing for allelism between lethals. Pairs of heterozygotes for a given lethal can be identified by examining the first clutch of eggs. The males and females are separated and used to make reciprocal cross tests with similar pairs from another line. The second clutch is then examined for the presence or absence of a lethal segregation. This procedure enables one to examine for concordance in the morphological pattern of damage between homozygous lethal embryos from the cross of an individual lethal heterozygote within and between lines. A misleading result may be given by this method if a female, which has stored sperm from a previous impregnation, fails to copulate with the second male before the second clutch of eggs is laid. This would cause a spuriously positive result, but a check on this is given by making several such reciprocal pair tests. An advantage of handling the actual homozygous lethal embryos directly is that labour can be saved in making cross tests for allelism between lethals by cutting down crosses between lethals which are morphologically obviously dissimilar in effect.

1957

The results of cross-testing sixteen of the nineteen lethals isolated in the first analysis of the St Mary's colony is shown in Fig. 3. Crosses were made within each morphological group. All the lethals within the morphological group E/L.N, namely 1, 4, 12, 13, 24, 30, 36, 40B, 42A, and 47A, were found to be allelic and named lethal E/L1. Crosses between the separate lines were complicated by marked deleterious effects of this lethal on the viability of the heterozygotes. Similarly, all the lethals of morphological type E/L.ABN, namely 34, 38, 40A, 42B and 47B,

were found to be allelic and named lethal E/L2. Crosses were not carried out in all combinations. Three lethals were lost before cross tests could be made (Nos. 2, 16 and 20).

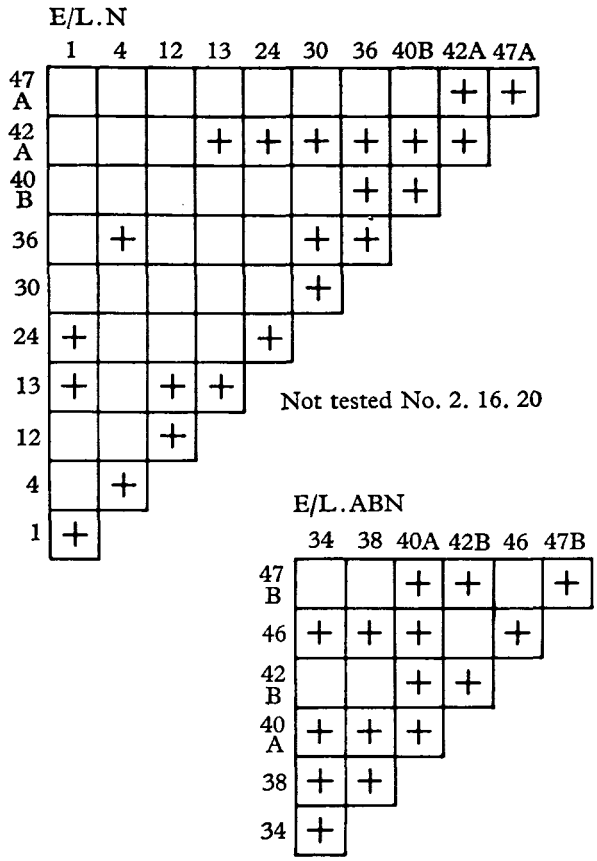

Fig. 3. Cross tests for allelism between lethals isolated from samples of third instar larvae taken in October, 1957. A (+) sign indicates allelic lethals, a (−) sign non-allelic lethals. Combinations not tested are left blank.

1958

Crosses in all combinations were made between the six lethals isolated in the second St Mary's analysis as shown in Fig. 4. Three lethals—namely 4A, 7A and 22—were alleles and named E/L3, and the other three lethals, 4B, 7B and 13, were also allelic and named E/L4.

In view of the morphological similarity of homozygotes for lethals E/L1 and E/L3 and of E/L2 and E/L4, cross tests were made to determine if these were at the same locus. Crosses were made in all combinations between these lethals and two others of independent origin. These were E1, a spontaneous laboratory lethal mutant, and E/L5, which was isolated in a small sample from a wrackbed colony

at Boulmer, Northumberland. The results are shown in Fig. 4. None of these lethals was found to be allelic to any other.

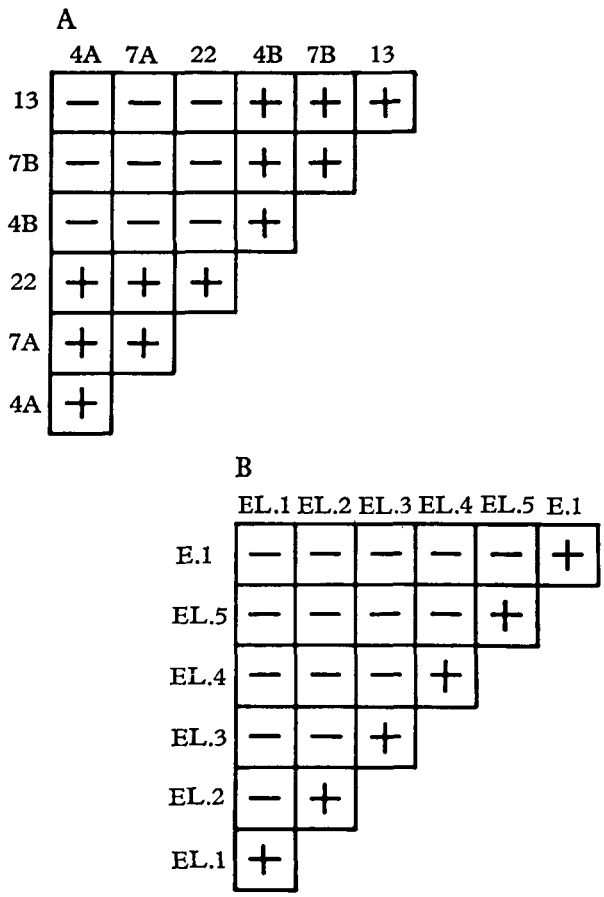

Fig. 4. A, cross tests for allelism between lethals isolated from samples of third instar larvae taken in April, 1958. B, cross tests for allelism between the lethals isolated in 1957 and 1958 and two other lethals of independant origin. For an explanation of the signs refer to fig. 3.

1959

The results of crosses between seventeen of the nineteen lethals isolated in the third analysis of the St Mary's colony are shown in Fig. 5. Crosses were carried out within two morphological groups. Lethals 1, 3B and 4 were found to be alleles and named E/L6. Lethals 3A, 37 and 39 were also alleles and named E/L7, and lethals 2 and 59 named E/L8. Three of the E-stage lethals, 6, 8 and 29, which were alleles were called E2. Two lethals were lost before cross tests could be made.

None of the repeated lethals in this analysis was allelic to any of the lethals isolated in the two previous analyses. The individual embryonic lethals identified by this method were found to be recessive autosomal factors with no morphological effects in the heterozygote.

https://doi.org/10.1017/S0016672300000732 Published online by Cambridge University Press

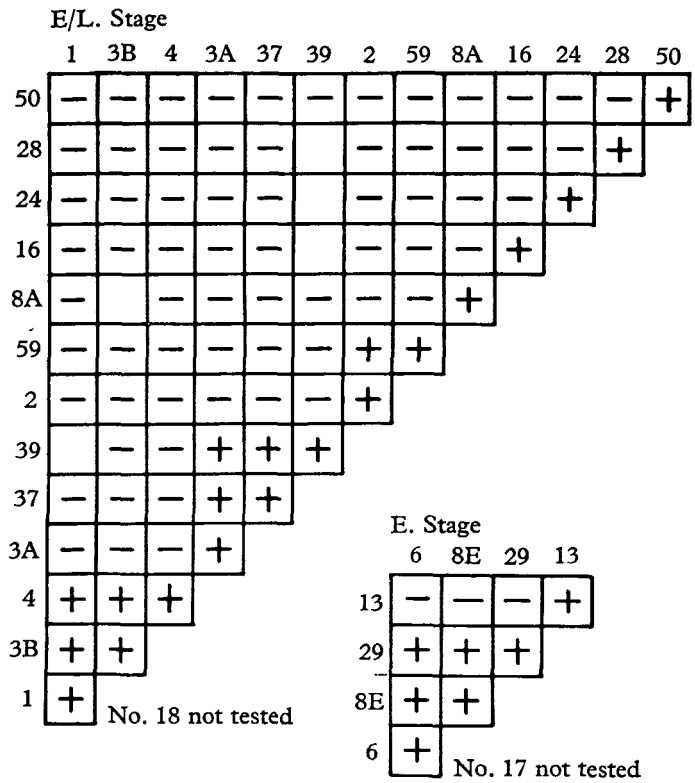

Fig. 5. Cross tests for allelism between lethals isolated from samples of third instar larvae taken in March-April, 1959.

Gene frequency estimates

Cross tests for allelism between lethals showed that certain lethals were isolated more than once in samples of larvae from the St Mary's colony, but the ecological analysis showed that the spatial dispersion of the larvae was patchy. These observations introduce the problem of error due to the method by which the population was sampled. In 1957 and 1958, groups of larvae were removed from the bed, whereas in 1959 larvae were taken singly. Table 7 shows that there is less repetition in 1959 than in the two previous sets of samples. Due to the tendency of members of a family to remain together, any two larvae sampled at the same position are likely to be consanguineous and will consequently increase the chances of sampling the same lethal more than once. This will bias any estimate of gene frequency which assumes the samples to have been drawn at random from the population. Calculations of gene frequency using the observed frequency of appearance of a lethal will give an overestimate due to error incurred in the sampling method, so that such an estimate of frequency must be taken as a maximum estimate. In order to eliminate the error due to sampling method we may discount repeats of a lethal within samples and count only repeats between samples, and so arrive at an unbiased estimate of the frequency of appearance of lethals within

the population as a whole. This has been done in Table 7, which shows the actual number of repeated lethals in the population after correction to be much lower than was apparent on first inspection of the data.

Table 7. *The frequency of appearance of lethals in samples of larvae from the St Mary's colony*

		Frequency of appearance				
Before correction	No. of lethals	1	2	3	...6	...10
1957	19	3*			1	1
1958	6			2		
1959	19	8*	1	3		

		Frequency of appearance		
After correction	No. of lethals	1	2	3
1957	7	4*		1
1958	2	2		
1959	15	9*	3	

* Including lethals not tested for allelism.

Gross frequency of embryonic lethals

First let us examine the effect of correction on the estimates of gross frequency of embryonic lethals in the population. The gross frequencies before correction are shown in Table 6. The three estimates are closely similar for the three years ($\chi^2_2 = 1{\cdot}52$, $P = 0{\cdot}5$). Gross frequencies after correction shown in Table 8 agree rather less well ($\chi^2_2 = 3{\cdot}98$, $P = 0{\cdot}15$) and are altogether lower than in the uncorrected series. The samples for 1957 were taken in October when the species is at its peak of seasonal abundance, whereas the samples for the following two years were taken in March and April when numbers are seasonally low (cf. larval density in Table 2). The gross frequency of embryonic lethals does not appear, therefore, to show any significant change associated with seasonal differences in population density.

Table 8. *Gross frequency of embryonic lethals in the St Mary's colony after correction for sampling method*

	Corrected number of lethals	Gross frequency
1957	7	0·0365
1958	2	0·0192
1959	15	0·0646

Frequency of individual embryonic lethals

In Table 9 maximum and minimum estimates of gene frequency have been calculated for individual lethals isolated more than once from the population; these are based on their frequency of appearance in samples before and after

https://doi.org/10.1017/S0016672300000732 Published online by Cambridge University Press

correction. The differences between the maximum and minimum estimates of gene frequency clearly illustrate the necessity for detailed information about the ecological structure of a population to assess the effectiveness of sampling methods employed for making estimates of gene frequencies.

Table 9. *The frequency of individual embryonic lethals in the St Mary's colony*

		Frequency of appearance		Gene frequency	
	Lethal	Observed	Corrected	Maximum	Minimum
1957	E/L1	10	3	0·05208	0·01563
	E/L2	6	1	0·03125	—
1958	E/L3	3	1	0·02885	—
	E/L4	3	1	0·02885	—
1959	E/L6	3	1	0·01293	—
	E/L7	3	2	0·01293	0·00862
	E/L8	2	2	0·00862	0·00862
	E2	3	2	0·01293	0·00862

The range of gene frequency estimates in Table 9 now raises the question: at what frequency does one expect to find an individual recessive lethal in a natural population? This problem has been investigated by Wright (1937). Wright has shown that for a completely recessive lethal with no selective disadvantage in the heterozygote, the distribution of gene frequencies is given by:

$$\phi(q) = c(1-q^2)^{2N} q^{4Nv-1}(1-q)^{-1} \tag{2}$$

$$\bar{q} = \frac{\Gamma(2Nv+\frac{1}{2})}{\sqrt{(2N)}.\Gamma(2Nv)}, \tag{3}$$

where q is the frequency of the lethal, N is the effective size of the population, and v the mutation rate to the lethal. From equation (3) we see that the mean frequency of a completely recessive lethal may be expressed in terms of the mutation rate and the population size. In order to estimate the expected frequency it is necessary to assign values to both of these terms.

As mentioned above, the St Mary's colony fluctuates in size, so it is necessary to decide what value of N to use. Where there is fluctuation in the size of a population, the effective number over a series of generations can be shown to be proportional to the harmonic mean of the series (Wright, 1939; Crow, 1954). In effect, what this means is that the effective size of the population is governed by its smallest phase of numbers. In the case of the St Mary's population, which reaches a minimum every alternate generation, the effective size of the population must be very close to the number of adult flies forming the foundation population at the start of each cycle. The population size at this point is shown in Table 1, giving $N = 1368$ individuals for the population on which the genetic analysis for 1959 was conducted.

No estimate of the mutation rate for individual embryonic recessive lethals has been made for *C. frigida*. However, estimates of the spontaneous mutation rate for an average lethal locus in three species of *Drosophila* made by different investigators turn out to be very similar, as shown in Table 10. *Coelopa*, like *Drosophila*,

Table 10. *Spontaneous mutation rate for an average lethal locus in three species of* Drosophila

Species	Chromosome	Average rate	Source
D. melanogaster	second	$2{\cdot}6 \times 10^{-5}$	Ives, 1945
D. melanogaster	second	$3{\cdot}1 \times 10^{-5}$	Dubinin, 1946*
D melanogaster	second	$1{\cdot}07 \times 10^{-5}$	Wallace, 1950
D. willistoni	second	$2{\cdot}2 \times 10^{-5}$	Dobzhansky, 1953
D. pseudoobscura	third	$1{\cdot}08 \times 10^{-5}$	Wright, Dobzhansky & Hovanitz, 1942

* Mean value calculated from the gross rate for the second chromosome (given by Berg, Dubinin & Olenov; listed in Dubinin) divided by the number of potentially lethal loci calculated by Ives (1945).

is an acalypterate fly with a very similar life-history and we may assume that the mutation rate for an average lethal locus is of similar magnitude. Using equation (3) the mean lethal gene frequency in a population of 1368 individuals has been calculated in Table 11. In fact, in approximately 80 % of colonies the lethal would not be present at all, and the remaining percentage of colonies are expected to show a range of frequencies, the mean ($\bar{q}$) being that for all colonies including those in which the lethal is absent. The estimates in Table 11 are considerably lower than the observed minimum frequency estimates for the repeated lethals of 1959 shown in Table 9. There are three possible reasons for this discrepancy which we may examine in turn.

Table 11. *The expected mean gene frequency for a recessive lethal in a population with* $N = 1368$, *for a range of values of mutation rate*

Mutation rate, v	Gene frequency, $\bar{q}$
1×10^{-5}	0·00087
$1{\cdot}5 \times 10^{-5}$	0·00128
$2{\cdot}0 \times 10^{-5}$	0·00173
$2{\cdot}5 \times 10^{-5}$	0·00213
$3{\cdot}0 \times 10^{-5}$	0·00245

In making the estimates of the expected mean gene frequency in Table 11 a range of values for v was chosen to correspond to the calculated rates for *Drosophila* species. A possible explanation of the discrepancy between the observed and expected lethal gene frequencies is that in *Coelopa frigida* the average rate is higher than in these *Drosophila* species. In fact, with a rate of $v = 1 \times 10^{-4}$ the expected frequency is $\bar{q} = 0{\cdot}00893$, very close to the minimum frequency estimates of 1959. Again, let us look at the indirect evidence from other species. The average mutation rate based on twenty-one estimates for individual deleterious loci in man listed by Penrose (1956) works out at $2{\cdot}6 \times 10^{-5}$, remarkably close to the

values obtained for *Drosophila*. Values obtained by Stadler (1942) for eight individual loci in maize vary widely from $4{\cdot}9 \times 10^{-4}$ to 1×10^{-6}. The values obtained for micro-organisms by several authors listed by Dobzhansky (1953) are much lower than this, mostly in the range 10^{-7} to 10^{-9}. Thus the indirect evidence from other species suggests that an *average* rate in the region of 1×10^{-4} for lethal loci in *Coelopa frigida* would be unusually high.

Where the adaptive value of the heterozygotes for a lethal is greater than that of the homozygous wild type a state of balanced polymorphism may be attained. If s_1 is the selection coefficient of homozygous wild type and s_2 that of homozygous lethal (necessarily unit), there is equilibrium at $q = s_1/(s_1+s_2)$ (Wright, 1937). The value of s_1 necessary to maintain lethals E/L7, E/L8, and E2 at equilibrium at the observed frequency would be $0{\cdot}00867$, the same order as the gene frequency, but evidence for selection coefficients of this order of magnitude would be difficult to obtain experimentally. The alternative possibility, that of selection against the heterozygotes for lethals, must also be considered. If V is the gross rate of mutation to embryonic lethals in genomes free from lethals, and P the chance of allelism of two lethals, then the mean value of selection against all lethals, $\bar{s}$, is given by Dobzhansky and Wright (1941) as:

$$\bar{s} = \frac{V - PQ(1+V)}{Q(1+2V-2PQ^2(1+V)}. \tag{4}$$

Considering the lethals isolated in 1959, we have seven single lethals and three double lethals (Table 7), so that $Q = 0{\cdot}056$ and $P = 0{\cdot}0385$. Substituting in equation (4), $\bar{s} = 0$, only for the low value of $V = 0{\cdot}00012$. Remembering that V represents the gross rate of mutation per generation over all loci capable of producing embryonic lethals, the possibility of selection against heterozygotes for lethals in the St Mary's colony cannot be ignored.

Determination of the gene frequency of a recessive lethal in a natural population is a stochastic process, involving the mutation rate producing new lethals in the population and the rate of elimination of lethals by homozygosis. In addition, sampling errors from generation to generation will cause random variations in gene frequency away from the equilibrium set by these two opposing rates. Equation (3) describes the resulting distribution of gene frequencies in populations of a given effective size, and Wright (1937, 1948) has shown that, in populations of the order of a thousand or so in size, random variations will be important in determining the gene frequency at any instant. With the limited number of gametes tested in each of the population samples the lethals isolated are necessarily those with frequencies in the upper tail of the distribution of gene frequencies. A plausible explanation of the high frequencies of the three lethals in 1959 is that they are due to random variance in a population of small genetically effective size.

The effective size of the population

If we assume that the effective size of the St Mary's population was equivalent to the observed census size of 1368 individuals, shifts in frequency of recessive

lethals as great as observed must from time to time occur due to random variance effects. However, it has been shown by Crow (1954), that the effective size of a population will be identical to the census size only under certain conditions, when the natural population approximates to an ideal Mendelian population. According to Crow's definition, in such a population there is: random mating, constant population size, equal numbers of both sexes, and a Poisson distribution of progeny number. In fact, few natural populations may be expected to conform to all these requirements.

In the case of *C. frigida* the population size fluctuates due both to seasonal changes and to the effects of the sea on the population habitat. We have already taken account of this by basing the calculations on the number of individuals at the beginning of the population cycle. Numerical inequality of the sexes frequently occurs in the wild, as shown in Table 1 and by the data of Egglishaw (1960). The problem of non-Poisson variation in progeny number is a difficult one for direct analysis. Counts on 929 wild females gave a mean of 76 eggs per clutch for *C. frigida* and a variance of 962, showing that there is non-Poisson variation in clutch size. However, there are two difficulties in the way of assessing the potential reduction in size of the effective breeding unit from this cause. Firstly, although a female may lay three or four clutches, we do not know the mean and variance of the number of clutches per female in the natural population. Secondly, we do not know the exact system of progeny survival. The fact that the eggs are laid as clutches, combined with the evidence from studies on spatial dispersion of the larvae, suggests that there may be a considerable positive intra-brood survival correlation. It is possible to calculate the maximum size of the effective unit by introducing the simplifying assumption that the number of clutches per female in the wild is randomly distributed, so that the effective unit is influenced by non-Poisson variation in clutch size alone. Crow & Morton (1954) have described the procedure for estimating from counts at a juvenile stage the variance in progeny number at maturity. The effective breeding unit is given by:

$$N_e = \frac{2N}{1 + s^2/\bar{x}} \tag{5}$$

where N is the number of parents, and $\bar{x}$ and s^2 are the mean and variance of progeny number at maturity (Crow, 1954). Table 12 shows the proportional reduction of the effective breeding unit expressed as the ratio of the census and effective numbers in a population of constant size due to non-Poisson variation in clutch size. In the first case, with a system of random survival from egg to adult, the reduction of the effective unit is comparable to the results of Crow & Morton (1954) for *Drosophila*, *Limnaea* and Man. For a system of complete family unit survival, that is with a positive intra-brood survival correlation of unity, the effective number would be very small. However, for a complex survival pattern in which, say, half the eggs were lost as entire clutches, followed by random survival for the rest of development, the effective number would be about half the census number.

Table 12. *The discrepancy between the census and effective sizes of a population of* C. frigida *due to non-Poisson variation in clutch size under three patterns of progeny survival*

	Complete random survival	Half eggs lost as entire clutches, and random survival for rest of development	Complete family unit survival
N_e/N	0·87	0·45	0·0007

On general grounds then, we may suspect that the effective breeding unit for the St Mary's population is rather less than the census numbers indicate. If this is so, the expected lethal gene frequencies in Table 11 will be overestimates. On the other hand, if the effective breeding unit is smaller than we have assumed, this would increase the probability of random variations in lethal gene frequencies in the population.

DISCUSSION

Local variations in the habitat of an animal species may be expected to lead to unevenness in its spatial distribution. The uneven distribution of the *Coelopa* larvae is the result of selection by the adult flies of oviposition sites in high-temperature zones which clearly ensures that the larvae hatch in portions of the bed where softening of the *Laminaria* is most rapid. The formation of aggregates within each temperature zone is probably the result of the feeding habits of the larvae. Ulyett (1949, p. 105) has found that congregation of blowfly larvae ensures a plentiful supply of salivary secretions for predigestion of the meat on which they feed. Single or small numbers of larvae are unable to concentrate sufficient secretion to exploit the food successfully. Some indication, though statistically non-significant, of a similar effect of underpopulation was found by Burnet & Thompson (1960) for *C. frigida*, but the *Laminaria* stipe in these experiments was split open to facilitate access by the larvae to the soft medulla, unlike the situation in the wild, so that any effect of underpopulation would have been minimized. It is probable that the formation of aggregates of larvae is an adaptation for the most efficient exploitation of the food medium.

The relevance of information of this kind to genetic analysis of wild populations lies in the effect of the underlying pattern of spatial dispersion on the content of samples taken for examination for the occurrence of mutants. This is likely to be more important where samples consist of relatively immobile juvenile stages than with more mobile adult flies. Similar observations using samples of juvenile stages from natural populations of other Diptera should provide an interesting comparative study. Ives (1945) found that the frequency of repetition of individual lethals in a sample of larvae taken from rotting fruit under a tree in an orchard population of *Drosophila melanogaster* in Florida was no greater than in a sample of mobile adult flies taken at the same place. Ives concluded that the females of *D. melanogaster* do not lay more than a few eggs at any one site. Compared with the high

frequency of repeated lethals before correction in the data for *Coelopa frigida* we have the sort of difference which might be expected between a species which lays its eggs together in a clutch on one oviposition site and one which lays its eggs one at a time and which may distribute them at a number of sites.

Nevertheless, the frequency of repetition of lethals in samples studied by Ives, from Maine, is rather high. But let us examine the method by which these samples were obtained. In the Maine collection four trap bottles were exposed for 14 hours at a seashore refuse dump. In this collection a few 'old-looking flies' were collected with a number of F_1 larvae. The genetic analysis was conducted on a sample of the F_1 and F_2 progeny of these individuals. Such a procedure introduces the risk of a high degree of consanguinity between individuals used for the genetic analysis, and if no correction is applied greatly increases the likelihood of isolating the same lethal more than once. Ives specifically states that the most desirable method for sampling a natural population is to take samples from as small an area as possible. Such a method of sampling is in my view to be avoided, since samples taken in this way cannot be regarded as being drawn randomly and fail to give an overall picture of the population under study. The results which have been obtained with *C. frigida* show that assessment of the frequency of individual lethals within a population must take account of the relationship of the method of sampling to the pattern of spatial dispersion of the stage in the life history being sampled. It is the distribution of lethals in the population as a whole that reflects the properties of its breeding structure, so that samples must be taken at as many positions as possible throughout the population. Moreover, we must not be too hasty in attributing special significance to repetition of individual lethals until we have examined the way in which they were drawn from the population under study.

It is not possible to do more than arrive at tentative conclusions regarding the frequency of embryonic lethals in the St Mary's colony until we have more information about the lethal mutation rate in *C. frigida*. Positive selection favouring lethal heterozygotes may be the correct explanation of the high frequencies of the three lethals in 1959, but if these are selectively maintained at high frequency in the population one would expect to have encountered them in the samples for the two previous years. The selective advantage of heterozygotes for deleterious recessive genes are not unknown in natural populations: there is, for example, the well-known case of the sickling trait in man (Allison, 1954), and the Brachyury alleles in wild populations of the mouse (Dunn & Suckling, 1956). Recently Terumi and Burdick (1959) have described a remarkable case of single gene heterosis associated with a second chromosome recessive lethal in a laboratory population of *Drosophila melanogaster*. On the other hand, an overwhelming body of evidence is accumulating from natural and laboratory populations which shows that, in general, supposedly recessive lethal genes have mildly deleterious effects on the viability of the heterozygous carriers (Dubinin 1946; Dobzhansky & Wright, 1941; Wallace, 1950; Stern *et al.*, 1952; Hiraizumi & Crow, 1960). Incompletely recessive effects on viability of certain lethals in the heterozygotes have also been detected in *Coelopa frigida* and will be reported separately.

Dubinin (1946) has produced evidence of changes in the gross frequency of recessive lethals in Russian populations of *Drosophila melanogaster* which he attributes to seasonal changes in population size. However, Goldschmidt *et al* (1955), studying populations of the same species in Israel, have been unable to confirm this 'Dubinin effect'. On the other hand, in the more recent data of Hiraizumi & Crow (1960) on American populations of this species, there is again evidence of seasonal change in the gross frequency of recessive lethals. The results which have been obtained with *Coelopa frigida* fail to show any difference in the frequency of recessive lethals at different periods in the seasonal cycle. As Goldschmidt *et al.* have suggested, the 'Dubinin effect' requires more detailed study.

Dobzhansky & Wright (1941), Dubinin (1946) and Paik (1960) found evidence of a high frequency of repetition of individual lethals in natural populations of *Drosophila* which they attribute to random variance in populations of small effective size. Similarly, Lewontin & Dunn (1960) have produced evidence to suggest that random variance effects are responsible for differences in the frequency of the Brachyury alleles in natural populations of the mouse. Certain lethals also occur repeatedly in the St Mary's population of *Coelopa frigida*, but on the available evidence it is not possible to state with certainty that this is due to random variance effects. On balance, however, this seems the most reasonable explanation.

SUMMARY

An account is given of the habitat and ecology of a wrackbed population of *Coelopa frigida* (Fab.). The spatial distribution of the third-instar larvae was found to be patchy. This is due to a preference of adult flies for oviposition sites in zones of high temperature within the wrackbed. The relationship between larval density and temperature was found to account only in part for the patchy distribution, a second factor being the formation of family aggregates within each temperature zone. The effect of this type of spatial distribution on the interpretation of genetic sampling methods for the population is discussed.

Samples of third-instar larvae from the wrackbed population have been analysed for the presence of recessive embryonic lethals in three consecutive years from 1957 to 1959. From a total of 528 gametes tested, 44 recessive embryonic lethals were isolated. Cross tests for allelism showed that certain lethals were repeated within samples taken in the same year.

The gross frequency of embryonic lethals is shown to be independent of seasonal changes in population density. Estimates of gene frequency are made for certain individual lethals and these are shown to be greater than expected on the basis of the census size of the population. Possible causes of this discrepancy are examined.

This work was carried out during tenure of a post-graduate studentship from the Department of Scientific and Industrial Research. The author wishes to express his gratitude to Dr U. Philip for her guidance throughout the course of this work and to Professor Sewall Wright and Dr Forbes W. Robertson for helpful suggestions during preparation of the manuscript.

REFERENCES

ALLISON, A. C. (1954). Protection afforded by sickle-cell trait against subtertian malarial infection. *Brit. med. J.* **1**, 290–294

ANSCOMBE, F. J. (1948). The transformation of Poisson, Binomial and Negative Binomial data. *Biometrica*, **35**, 246–254.

BACKLUND, H. O. (1945). The wrack fauna of Sweden and Finland. Opusc. ent., Suppl. V.

BLACK, W. A. P. (1948). The seasonal variation in chemical composition of some sub-littoral seaweeds common to Scotland. *J. Soc. chem. Ind., Lond.*, **69**, 165–168, 172–176.

BURNET, B. (1960). The European species of the genus *Coelopa* (Dipt., Coelopidae). *Ent. mon. Mag.* **96**, 8–13.

BURNET, B. (1960). The larvae of *Coelopa frigida* (F.) and *Coelopa pilipes* (Hal.). *Ent. mon. Mag.* **96**, 178–180.

BURNET, B. & THOMPSON, U. (1960). Laboratory culture of *Coelopa frigida* (Fabricius) (Diptera: Coelopidae). *Proc. R. ent. Soc. Lond.* A, **35**, 85–89.

CROW, J. F. (1954). Breeding structure of populations. II: Effective population number. In *Statistics and Mathematics in Biology*, ed. Kempthorn *et al.* Iowa, S.P.

CROW, J. F. & MORTON, N. E. (1954). Measurement of gene frequency drift in small populations. *Evolution*, **9**, 202–214.

DOBZHANSKY, TH. (1953). *Genetics and the Origin of Species*. Columbia University Press.

DOBZHANSKY, TH., PAVLOVSKY, O., SPASSKY, D. & SPASSKY, N. (1955). Genetics of natural populations. XXIII: Biological role of deleterious recessives in populations of *Drosophila pseudoobscura*. *Genetics*, **40**, 781–796.

DOBZHANSKY, TH. & WRIGHT, S. (1941). Genetics of natural populations. V: Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. *Genetics*, **26**, 23–51.

DUBININ, N. P. (1946). On lethal mutations in natural populations. *Genetics*, **31**, 21–38.

DUNN, L. C. & SUCKLING, J. (1956). Studies of genetic variability in populations of wild house mice. I: Analysis of seven alleles at locus T. *Genetics*, **41**, 344–352.

EGGLISHAW, H. J. (1960). Studies on the family Coelopidae (Diptera). *Trans. R. ent. Soc. Lond.* **112**, 109–140.

EVANS, D. A. (1953). Experimental evidence concerning contagious distributions in ecology. *Biometrica*, **40**, 186–211.

GOLDSCHMIDT, E., WAHRMAN, J., LEDERMAN-KLEIN, A. & WEISS, R. (1955). A two years' survey of population dynamics in *Drosophila melanogaster*. *Evolution*, **9**, 353–366.

HADORN, E. (1951). Developmental action of lethal factors in *Drosophila*. *Advanc. Genet.* **4**, 53–85.

HIRAIZUMI, Y. & CROW, J. F. (1960). Heterozygous effects on viability, fertility, rate of development, and longevity of *Drosophila* chromosomes that are lethal when homozygous. *Genetics*, **45**, 1071–1083.

IVES, P. T. (1945). Genetic structure of American populations of *Drosophila melanogaster*. *Genetics*, **30**, 167–195.

LEWONTIN, R. C. & DUNN, L. C. (1960). The evolutionary dynamics of a polymorphism in the house mouse. *Genetics*, **45**, 705–722.

MACLEOD, J. & DONNELLY, J. (1947). Individual and group marking methods for fly-population studies. *Bull. ent. Res.* **48**, 485–92.

PAIK, Y. K. (1960). Genetic variability in Korean populations of *Drosophila melanogaster*. *Evolution*, **14**, 293–303

PAVAN, C., CORDIERO, A. R., DOBZHANSKY, N., DOBZHANSKY, TH., MALAGOLOWKIN, C., SPASSKY, B. & WEDEL, M. (1951). Concealed genic variability in Brazilian populations of *Drosophila willistoni*. *Genetics*, **36**, 13–30.

PENROSE, L. S. (1956). The spontaneous mutation rate in Man. *Brit. Med. Res. Coun. Rep.*, H.M.S.O., Cmd. 9780, pp. 90–92.

QUENOUILLE, M. H. (1950). *Introductory Statistics*. London: Butterworth-Springer, Ltd.

SPENCER, W. (1947). Mutations in wild populations of *Drosophila*. *Advanc. Genet.* **1**, 359–401.

STADLER, L. J. (1942). Some observations on gene variability and spontaneous mutation. *Spragg Mem. Lectures*, **3**, 3–15.

STERN, C., CARSON, G., KINST, M., NOVITSKI, E. & UPHOFF, D. (1952). The viability of heterozygotes for lethals. *Genetics*, **37**, 413–449.

SUTCLIFFE, D. W. (1960). Osmotic regulation in the larvae of some euryhaline Dipera. *Nature, Lond.*, **187**, 331–332.

TERUMI, M. & BURDICK, A. B. (1959). Single gene heterosis associated with a second chromosome recessive lethal in *Drosophila melanogaster*. *Genetics*, **44**, 211–232.

WALLACE, B. (1950). Autosomal lethals in experimental populations of *Drosophila melanogaster*. *Evolution*, **4**, 172–174.

WRIGHT, S. (1937). The distribution of gene frequencies in populations. *Proc. nat. Acad. Sci., Wash.*, **23**, 307–320.

WRIGHT, S. (1939). Statistical genetics in relation to evolution. *Actualités scientifiques et industrielles*, No. 802. Paris: Hermann et Cie.

WRIGHT, S. (1948). On the role of directed and random changes in gene frequency in the genetics of populations. *Evolution*, **1–2**, 279–294.

WRIGHT, S., DOBZHANSKY, TH. & HOVANITZ, W. (1942). Genetics of natural populations. VII: The allelism of lethals in the third chromosome of *Drosophila pseudoobscura*. *Genetics*, **27**, 363–394.

ULYETT, G. C. (1949). On competition for food and allied phenomena in sheep blowfly populations. *Phil. Trans.* B, **234**, 77–173.