

THE GENETICS OF *DROSOPHILA SUBOBSCURA* POPULATIONS

XV. EFFECTIVE SIZE OF A NATURAL POPULATION ESTIMATED BY THREE INDEPENDENT METHODS

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SUMMARY

An estimate is presented of the effective size of a natural, Greek population of *Drosophila subobscura*, based on data on lethal allelism. It is compared with further estimates for the same population obtained from newly-reported data by the "temporal" and the "ecological" methods. The lethal allelism and ecological estimates both suggest that the population is extremely large and effectively infinite. The conclusions from the temporal method conform to this, with the exception of data from two loci: *Est-5* and *Aph*. Directional selection at these loci is therefore suggested. Each method is described and explained, and their validity discussed. The results are compared with the few previous estimates of effective population size.

1. INTRODUCTION

POPULATION genetics theories, in general, deal with "ideal" populations, which are closed, of constant size, have discrete generations, random mating (including self-fertilisation at random), and a random distribution of family sizes. Effective population number, N_e —a concept developed by Wright (1931, 1938)—relates theory to practice, and, essentially, is the size of the ideal population with which an actual population can be equated genetically. Thus Wright (1969) described the estimation of N_e as "... a practical necessity in dealing with natural populations": an estimation of N_e should precede the application of population genetics theories to natural populations. In practice, however, it is common for geneticists to postulate or implicitly assume that N_e is sufficiently large to be ignored. Such a procedure, no doubt, would often be justified by the (regrettably unknown) facts. Yet it is clearly desirable to replace postulates and assumptions with actual measurements.

There are two broad approaches to the estimation of effective population number. The first approach—within which several methods are subsumed—makes use of genetic information, but generally rests on assumptions which cannot be tested directly. The second approach utilizes ecological information, but requires certain key measurements—concerning inter-population migrants and the distribution of family sizes—which are difficult to obtain from natural populations.

Loukas *et al.* (1980) utilized the lethal allelism method, first described by Dobzhansky and Wright (1941), to estimate the effective size of a natural

population of *Drosophila subobscura* from Mount Parnes, Greece. This population is located 40 km north of Athens in a fir woodland (*Abies cephalonica*) at an elevation of 900 m. In the present paper we summarize these lethal allelism results, and describe further attempts to measure the effective size of the same population by the "temporal method" (Krimbas and Tsakas, 1971) using electromorph frequencies, and by the ecological method (Begon, 1977). This appears to be the first thorough attempt to measure the effective size of the same population both genetically and ecologically, and this paper, therefore, has three main aims.

The first, quite simply, is to add to our rather poor knowledge of the effective size of natural populations. This is particularly important for a species like *D. subobscura*, for which there is a substantial body of genetic information. The second aim is to set out the details of the methods, and to state explicitly the assumptions on which each is based. The third is to compare the answers derived from these three quite separate and independent methods. Agreement will then suggest that each can be applied in future with increased confidence; while contradictory results will demand a reappraisal of each of the methods and of their inherent assumptions. In a sense therefore this comparison represents a test of these assumptions, since, if there is disagreement, some of them at least must be false.

2. ALLELISM OF LETHALS

The procedure for estimating effective population size in *D. subobscura* by the classical method based on the allelism of lethals (Dobzhansky and Wright, 1941; Wright *et al.*, 1942; Prout, 1954) has been fully described by Loukas *et al.* (1980). This, and the results obtained, will only be summarized briefly here.

O-chromosome lethals (and semi-lethals) were isolated from samples of flies collected from the Mount Parnes population, and from a population from the orange orchards of Alikianou (Chania, Crete). The allelism of these lethals, *i.e.* the extent to which the same loci are affected in different isolates, was tested by the appropriate crosses both within and between populations. The following simple argument illustrates how such data can furnish information on effective population size.

Two distinct populations (Mount Parnes and Alikianou, for instance) will accumulate recessive lethals independently. There is therefore only a low probability that a lethal carried by an individual from the first population will also be carried by an individual from the second: the rate of allelism will be low. *Within a small* population, however, there will be a high level of inbreeding, and two individuals from the population will have a comparatively high probability of carrying the same lethal (the rate of allelism will be high). As effective population size increases, this probability will decline; it will approach the between-population value as the effective size approaches infinity. A comparison of the rate of allelism between and within populations, therefore, will provide an estimate of effective population size. It should be noted, however, that such an argument is based on the following implicit assumption: that the mutation-rate to lethal alleles is equal at different loci.

The results obtained by Loukas *et al.* (1980) for rates of allelism of lethal genes (along with their 95 per cent confidence limits) were as follows:

within Mount Parnes:	0.0075 (0.0022–0.0143)
within Alikianou:	0.0061 (0.0001–0.0178)
between populations:	0.0078 (0.0035–0.0144)

These figures are not significantly different from one another, and therefore suggest that the Mount Parnes *D. subobscura* population is effectively infinite in size.

3. THE TEMPORAL METHOD

The “temporal method” has been presented by Krimbas and Tsakas (1971) and Lewontin and Krakauer (1973) as a test of selective neutrality, but it has not been fully described as a means of estimating N_e from data obtained from several loci. We take the opportunity to do so now.

We start from the assumption that the genic variation we are dealing with is selectively neutral. The basis of the method is then that the change in gene-frequency over time (due to drift) will be greatest when the effective population size is small, or, equivalently, when f_i , the “effective inbreeding coefficient”, is large. f_i , like N_e , being an “effective” parameter, accounts for the discrepancy between an actual population and the ideal population from which an “ordinary” inbreeding coefficient might be obtained. In fact, if we imagine an ensemble of imaginary populations at time t generations, all derived directly from the same population at time zero, then:

$$f_i = \frac{\sigma_p^2}{p_0(1-p_0)} \quad (\text{Wahlund, 1928})$$

where σ_p^2 is the variance in the frequency of an allele from population to population, and p_0 is its initial frequency.

σ_p^2 cannot be obtained directly, but $(\Delta p)^2$ —the square of the change in frequency over time in a single population—is an estimate of it with one degree of freedom (Krimbas and Tsakas, 1971; Lewontin and Krakauer, 1973). Thus f_i can be estimated:

$$\hat{f}_i = \frac{(\Delta p)^2}{p_0(1-p_0)}$$

Of course, this relationship should hold for all n alleles at a locus, so that a better estimate is obtained by taking a mean:

$$\bar{f}_i = \frac{1}{n} \sum_{i=1}^{i=n} \frac{(\Delta p)^2}{p_{0i}(1-p_{0i})}$$

There is no doubt, however, that the apparent change in allele-frequency will also be affected by sampling error on the part of the investigators. Thus, following Krimbas and Tsakas (1971), we should subtract a “correction factor” from \bar{f}_i . This can be derived by imagining that the change in frequency is due to sampling error alone, in which case Δp will be approximately equal to its own standard error. Sampling error alone, therefore, can be expected to give:

$$\bar{f}_i = \frac{1}{M_1} + \frac{1}{M_2}$$

where M_1 and M_2 are the numbers of genes examined on the first and second occasions, and a better estimate of f_i is:

$$\tilde{f}_i = \tilde{f}_i - \left(\frac{1}{M_1} + \frac{1}{M_2} \right).$$

Finally, it will be apparent that this value should be the same for every locus, and that a better estimate still will be obtained by deriving a mean from all l loci:

$$\hat{f}_i = \frac{1}{l} \sum_{j=1}^{j=l} \tilde{f}_{ij}$$

Effective population number can then be obtained from the equation:

$$\hat{f}_i = 1 - \left(1 - \frac{1}{2\hat{N}_e} \right)^t \quad (\text{Falconer, 1970}).$$

The standard error of \hat{N}_e has been derived by Lewontin and Krakauer (1973), using the fact that the underlying distribution of Δp is binomial, and is:

$$SE_{\hat{N}_e} = \hat{N}_e \sqrt{2/m}$$

where m is the number of independent allelic proportions under consideration.

Clearly, this method ought to give reliable estimates of N_e if the genic variation is selectively neutral. On the other hand, changes in gene-frequency mediated by directional selection will inflate Δp , and therefore lead to an underestimate of N_e ; while stabilising selection will lead to small Δp 's and an overestimate of N_e . In fact, the method was originally conceived as a test of selective neutrality: drift should affect all loci similarly; whereas selection is unlikely to do so, and should result in heterogeneous estimates of f_i (and N_e).

Samples were taken from the Mount Parnes population for the examination of electromorph frequencies on three occasions: 23 September 1975, 15 September 1976 and 23 May 1977. On each occasion the following loci were examined: *Est-3*, *Est-5*, *Est-7*, *Aph*, *Pgm*, *Hk 1*, *ME*, *Xdh* and *AO*. These loci were chosen because they showed no linkage disequilibrium, either between one another or with chromosomal inversions (Loukas, Krimbas and Vergini, 1979); had they done so, the f_i -values would have been artificially homogenised. Further details of the methods for examining these loci in *D. subobscura* may be found in Loukas, Krimbas, Mavragani-Tsipidou and Kastritis (1979) and Loukas and Krimbas (1980). As we shall argue in the section dealing with the ecological method, there appear to be seven generations separating the first two samples, and two generations between the second and third. The results are presented in table 1. Estimates of N_e (and f_1) have been derived for both time periods, and individual estimates of f_1 have been computed for each locus over each time period. The two estimates of N_e quite obviously disagree: the one relating to the whole year from September 1975 to September 1976 is 268 (standard error: 73); while the one relating to the winter (bottleneck) population is infinity. It is clear, however, that the discrepancy is due to the results at just

TABLE 1

Allelic frequencies at nine loci, and the effective inbreeding coefficients and effective population sizes estimated from them by the temporal method. For further explanations see text

Locus	Allele	Allelic frequencies at time, t (generations)			Effective inbreeding coefficients	
		$t = 0$	$t = 7$	$t = 9$	$\hat{f}_1^{(0-7)}$	$\hat{f}_1^{(7-9)}$
Sample size:		190	250	335		
<i>Est-3</i>	1.20	0.289	0.325	0.255	-0.00001	0.0035
	1.00	0.674	0.621	0.689		
	0.90	0.037	0.054	0.056		
<i>Est-5</i>	1.25	0.000	0.016	0.011	0.0101	0.0019
	1.06	0.026	0.068	0.057		
	1.00	0.405	0.300	0.374		
	0.90	0.527	0.523	0.503		
	0.86	0.021	0.093	0.049		
	0.78	0.021	0.000	0.006		
<i>Est-7</i>	1.04	0.058	0.029	0.047	0.0006	0.0001
	1.02	0.010	0.003	0.000		
	1.00	0.932	0.968	0.953		
<i>Aph</i>	1.27	0.000	0.010	0.012	0.0079	-0.0020
	1.00	0.453	0.568	0.532		
	0.73	0.547	0.412	0.442		
	0.46	0.000	0.010	0.015		
<i>Pgm</i>	1.32	0.042	0.055	0.033	-0.0004	-0.0003
	1.10		0.940			
	1.00		0.930			
	0.71		0.015			
<i>Hk 1</i>	1.43	0.005	0.003	0.003	-0.0012	-0.0022
	1.27	0.400	0.385	0.405		
	1.00	0.492	0.509	0.473		
	0.73	0.103	0.103	0.119		
<i>ME</i>	1.10	0.016	0.015	0.013	-0.0013	-0.0007
	1.00	0.947	0.941	0.924		
	0.94	0.037	0.041	0.062		
	0.75					
<i>Xdh</i>	1.08	0.021	0.012	0.020	0.0007	-0.0007
	1.06		0.118			
	1.04		0.097			
	1.00		0.768			
	0.96		0.724			
	0.92		0.091			
	0.86		0.092			
<i>AO</i>	1.10	0.116	0.080	0.093	0.0004	-0.0023
	1.08	0.237	0.188	0.158		
	1.05					
	1.00					
	0.95					
	0.91					
	0.82					
Effective inbreeding coefficient, \hat{f}_1						
Effective population size, N_e (\pm standard error)				268 \pm 73	∞	

two loci: *Est-5* and *Aph*. If the data from these loci are ignored, then N_e for the first time period is also infinite. Moreover, if the absolute values of the \hat{f}_1 's derived from individual loci are plotted on a frequency histogram, then, as fig. 1 shows, 16 of the 18 values are clustered around zero ($N_e = \infty$), but

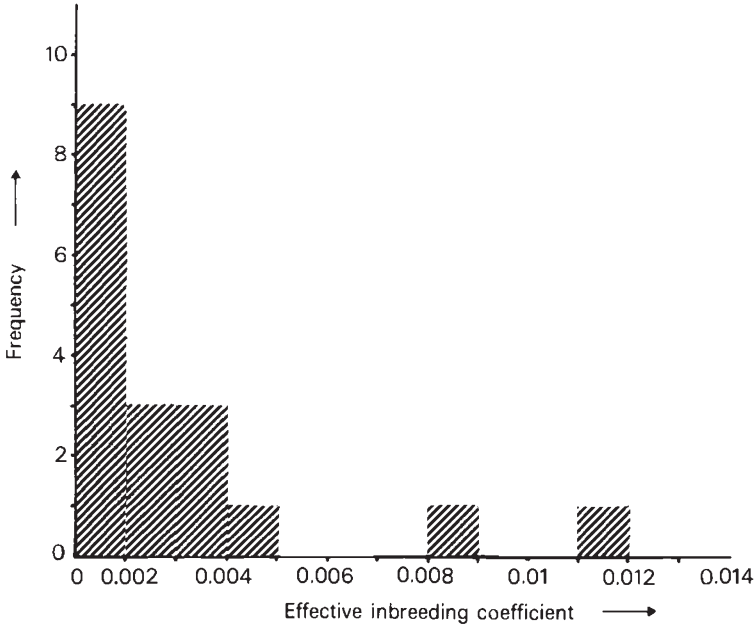


FIG 1. Frequency histogram of the moduli of the eighteen effective inbreeding coefficients from table 1.

the *Est-5* and *Aph* values stand well apart. And finally comparison of the \hat{f}_1 -estimate derived from the *Est-5* and *Aph* loci over both time periods with the estimate derived from the rest of the data, shows that the difference is significant at the 1 per cent level. These results will be discussed in the final section of the paper.

4. THE ECOLOGICAL METHOD

The accurate estimation of N_e by the ecological method requires data on the area of the panmictic circle (*sensu* Wright, 1969), the rate of migration between populations, the numbers of breeding males and females in each generation, the distribution of family sizes, and the pattern of mating. In practice, however, previous workers have obtained and used only a portion of these data. Some, for instance, have relied only on the approximate distribution of family sizes (Merrell, 1968; Tinkle, 1965); others have used only the area of the panmictic circle, and the density within it of a single generation (Crumpacker and Williams, 1973; Begon, 1976a; Loukas and Krimbas, 1979); Begon (1977) ignored mating pattern, while both Begon (1977) and Greenwood (1975) ignored inter-population migration, and relied on laboratory data for the distribution of family sizes. Nevertheless, it

should be appreciated that it is rarely necessary for the estimate to be reliable at a level more refined than the appropriate order of magnitude; and, in the present case, it is really only necessary to know if there is definite agreement or contradiction between the ecological and the other estimates.

The various facets of the ecological estimation will now be considered in turn, and a minimum estimate of N_e derived. This will be followed by a consideration of our "best" estimate of N_e .

The "panmictic circle" of a species is the area within which mating, in a single generation, could *potentially* be random. Its estimation requires data on dispersal and "life-span", combined in the formula:

$$\text{Area of panmictic circle} = \frac{4}{3}\pi S^2 T \quad (\text{Wright, 1969})$$

where S^2 is the distance variance of dispersed flies in a single activity period, and T is the number of activity periods over which an individual has some probability of producing offspring. *D. subobscura*'s daily rate of dispersal has been measured for populations in Switzerland (Burla and Greuter, 1959; Greuter, 1963), England (Begon, 1976a) and Greece (Loukas and Krimbas, 1979) (though not, unfortunately, for the Mount Parnes population), and there is good agreement between the results. In the present case the results from the nearest (Greek) population will be used. These are smaller than the estimates from Switzerland, but larger than those from England. More recent estimates from England, however, (Inglesfield, 1979) are also larger than those from Greece, so the estimates used here may well be conservative. If they are combined with the conventional (but almost certainly conservative) T -value for *Drosophila* of 11.5 days (Dobzhansky and Wright, 1943), then we have panmictic areas of 91,992 m² for males, and 80,581 m² for females.

The extent of the Mount Parnes population, as judged by habitat, is 10⁷ m² at the very least, and it is certain, therefore, that the flies of a single generation are not confined by their environment to an area smaller than the panmictic circle. When we consider successive generations, however, it is clear that individuals from one panmictic circle mix freely with individuals from adjoining areas, so that there is considerable migration between populations. Such migration has not been quantified, and it would be difficult to do so; but it will serve our present purpose to assume that there are no inter-population migrants. In other words, we will assume that the population within the panmictic circle is closed. We can then conclude unequivocally that, in this respect, we are underestimating N_e .

Collections of *D. subobscura* from Mount Parnes over the past decade suggest a thriving population of flies between early-May and late-September. In the present case, we report estimates of population density for two periods of the year; late-March/early-April and early-August. The physiological condition of the females collected in the former period was examined, and, as table 2 shows, there was an overwhelming preponderance of mature flies and of flies with deposits of fat in their abdomen. If these data are combined with meteorological data for the previous three months on Mount Parnes (fig. 2), and interpreted in the light of Begon's (1976b) study of reproductive condition in *D. subobscura*, then the conclusion is unavoidable that these are flies which have overwintered. The density of the population breeding in early-April is, therefore, the yearly minimum. The

TABLE 2

Reproductive condition and fat content of female flies collected 27.3.79 and 7.4.79

	Flies with undeveloped ovaries; newly-emerged	Flies with developing ovaries	Flies with ovaries with mature eggs
Adult fat deposits in the abdomen	0	10	41
No fat deposits	2	0	23

early-August population, on the other hand, is more typical of the populations breeding in the warmer part of the year.

Population density was estimated by capture-recapture on two occasions in early-August 1978, using the modification of Jackson's "negative" method described by Begon (1979). The study-site was centred on the point from which the flies used for genetic estimates had previously been caught, and was covered by a rectangular grid of traps (eight traps by four), spaced at approximately 16.5 m intervals. The traps themselves were plastic boxes, 100 mm × 100 mm × 110 mm, containing a bait of fermented peach. Each

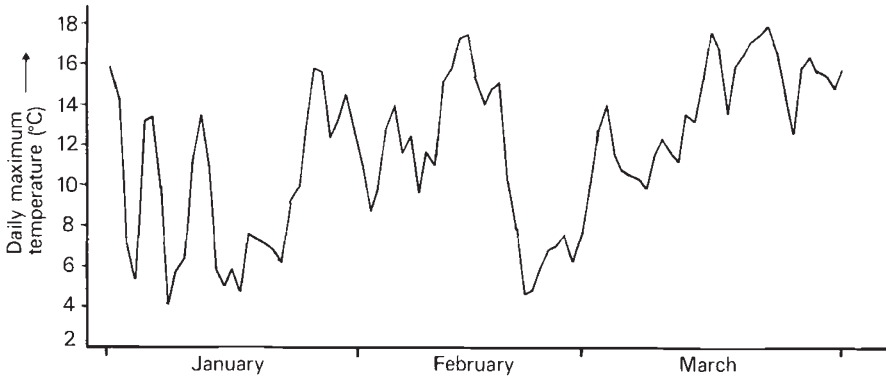


FIG 2.—Daily temperature maxima at the study-site (Mount Parnes) January–March 1979.

study lasted four days. On the first three days flies were trapped, and *obscura*-group *Drosophila* were counted, marked with micronized fluorescent dust and released; previous collections had established that at least 98 per cent of the *obscura*-group *Drosophila* on Mount Parnes were *D. subobscura*. On the fourth day the flies were caught and returned to the laboratory. There they were identified and the presence of marks noted. Further details of the handling and marking procedures can be found in Begon (1976a). It had been intended that males and females should be dealt with separately throughout. The large numbers caught, however, made separation in the field impracticable. Instead, it was assumed that the sex-ratio on the first three days was the same as that measured exactly on the fourth.

The results are shown in table 3. They present a reasonably consistent picture of around 4.5×10^4 flies on the study-site. A more accurate overall estimate can be obtained by calculating separate male and female means, weighted by the reciprocals of the squares of the individual standard errors.

TABLE 3
Capture-recapture data and estimates, July–August 1978

Final catch	31 July–3 August 1978		9–12 August 1978	
	♂♂	♀♀	♂♂	♀♀
Unmarked	2234	2765	1084	2134
Day 1—marked (Nos. released in brackets)	1(421)	4(529)	6(451)	13(876)
Day 2—marked (Nos. released in brackets)	15(651)	27(818)	17(529)	21(1026)
Day 3—marked (Nos. released in brackets)	39(980)	79(1230)	47(953)	70(1849)
Population size (±1 S.E.)	22,092 ±7446	18,095 ±4350	12,669 ±3498	36,442 ±8225
Daily survival-rate (±1 S.E.)	0.41 ±0.097	0.42 ±0.070	0.56 ±0.099	0.61 ±0.086

This suggests that the population on the study-site in early-August consisted of 14,373 males and 22,104 females. To convert these to densities we must make an assumption about the area beyond the grid from which traps attract flies; data on dispersal in *D. subobscura* from Begon (1976a) and Loukas and Krimbas (1979) both suggest that an extension of the grid of approximately 30 m in each direction is appropriate. Thus the total area of the study-site is 19,217 m²; the densities are: males 0.75/m², females 1.15/m²; and the numbers within the panmictic circle are: 68,994 males and 92,668 females. This is likely to be an underestimate, in that there is a gradient of decreasing catching efficiency beyond the peripheral traps, and the true area of the study site is probably smaller than suggested here.

If these male and female numbers are combined using the equation given by Crow and Kimura (1970), then:

$$N_e = \frac{4 \times \text{male numbers} \times \text{female numbers}}{\text{male numbers} + \text{female numbers}} = 158,195.$$

A further attempt to measure density was made during late-March and early-April 1979. During this period the catches were very small (due, to some extent, to the sub-optimal weather conditions), and so several rather approximate methods have been used. The first two of these concern an investigation carried out 25–27 March 1979. The study-site was approximately 1000 m from the usual collecting-site, but in a similar habitat, and at a similar elevation. On the first two days, known numbers of male and female laboratory-reared wild-type flies, marked with fluorescent dust, were released from a central point. On the third day, traps with fermented-banana bait were arranged in a series of four octagonal annuli around the release-point (fig. 3)—an arrangement described, and discussed by Crum-packer and Williams (1973) and Begon (1976a)—and the flies captured at each of the traps (table 4). The distance between traps in the lines radiating from the centre was 12.5 m, but because of the position of a road, the most peripheral trap of one of the lines was missing. Thus, with one trap at the central point, there were 32 traps in all.

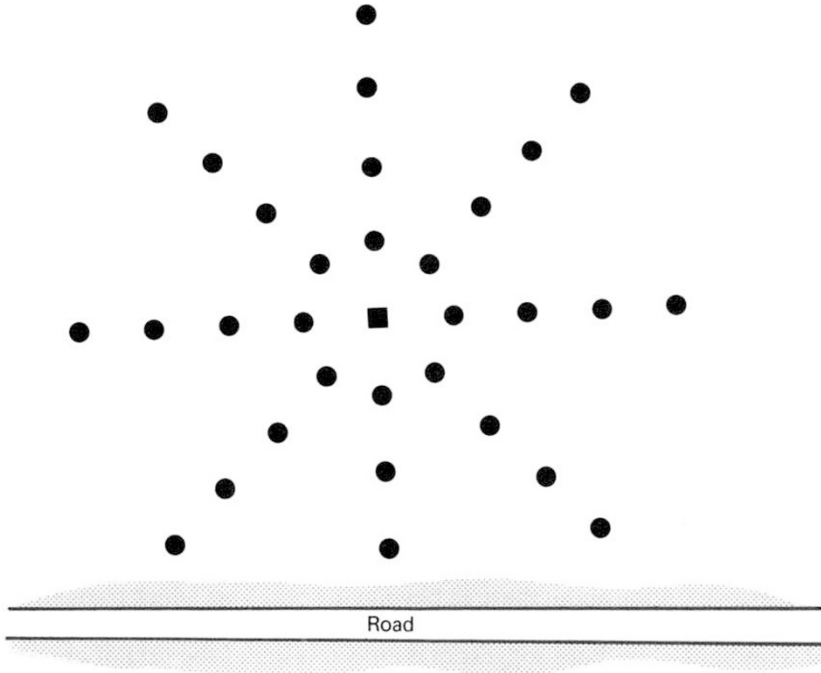


FIG 3.—Grid of traps, 27.3.79.

The arrangement of the traps means that, if flies are attracted to their nearest trap, then flies originally in an annulus will be attracted to a trap within the same annulus (Crumpacker and Williams, 1973). Thus, the flies caught in annuli 2, 1 and 0 (the centre trap) will all have come from these same annuli, and no flies from these annuli will have been caught elsewhere.

TABLE 4

Capture-recapture data and maximum density estimates, 25–27.3.79. For further explanation see text

Males					Females			
Released day 1 (yellow): 410					Released day 1 (yellow): 396			
Released day 2 (green): 422					Released day 2 (green): 430			
Recaptures					Recaptures			
Annulus	Area	Unmarked	Yellow	Green	Annulus	Unmarked	Yellow	Green
0	($a = 129.4 \text{ m}^2$)	3	27	13	0	1	30	22
1	$8a$	34	2	1	1	15	1	0
2	$16a$	77	0	0	2	24	0	0
3	$24a$	45	0	0	3	21	0	0
4	$32a$	50	0	0	4	27	0	0
Maximum densities* ($/\text{m}^2$)								
$D_{0G}: 0.35\phi$					$D_{0G}: 0.099\phi$			
$D_{0Y}: 0.34\phi$					$D_{0Y}: 0.100\phi$			
$D_{1G}: 0.32\phi$					$D_{1Y}: 0.089\phi$			
$D_{1Y}: 0.31\phi$								

* D_{0G} , for instance, means the estimate for annulus 0 using green marks

Since the efficiency of capture is highest in these traps (Begon, 1976a, Loukas and Krimbas, 1979), the numbers of (unmarked) flies caught may themselves be used to obtain a minimum estimate of density (using outer annuli would depress the estimate still further). In the present case, the minima computed are: males $0.034/m^2$, females $0.013/m^2$.

The second estimates are derived from a simple capture-recapture procedure, on the assumption that the efficiencies of capture of marked and unmarked flies are the same. Each annulus is treated separately, and the numbers of marked flies *available* for capture are estimated from the numbers actually caught in each annulus (for a fuller explanation see Crumpacker and Williams, 1973 and Begon, 1976a). Thus:

$$\hat{N}_i = n_i \times \frac{R}{m_i} \times \hat{q}_i \times \phi$$

where N_i is the total number of unmarked flies in annulus i , n_i and m_i are numbers of unmarked and marked flies caught in annulus i , R is the number of marked flies previously released, \hat{q}_i is the estimated proportion of the available marked flies that are in annulus i , and ϕ is the survival rate of marked flies against mortality. In fact, when marked flies of different colours are released on two consecutive days, ϕ can itself be estimated from the discrepancy between the numbers of "young" and "old" marks recaptured.

As table 4 shows, in our case there were considerably more old (yellow) flies recaptured than young (green). This was almost certainly due to the fact that the release of green-marked flies was immediately followed, not to say accompanied, by a period of heavy rain, which is likely to have killed a number of the newly-marked flies. An estimate of ϕ in such a case, would exceed unity—a biological impossibility—and so a modified procedure has been used. We shall assume that for yellow marks the survival-rate is ϕ , while for green marks it is ϕ multiplied by the ratio of green to yellow recaptures. This should take account of the post-marking mortality on day 2, but will mean that all estimates include ϕ as a multiplier. ϕ is unknown, but certainly less than unity, and in this sense the estimates presented in table 4 are maxima. Given the paucity of data they present a remarkably consistent picture, and the means lead to density estimates for males of $0.33/m^2$, and for females of $0.096/m^2$, which should, in each case, be multiplied by the appropriate ϕ -value.

Two further estimates of male and female density can be computed from another application of Jackson's "negative" method, on the original grid, from 4-7 April 1979. The first is an estimate derived simply from the method itself in the normal way; the second is obtained by multiplying the August 1978 estimates by the ratio—"number caught in April 1979: number caught in August 1978". This avoids the use of the low number of recaptures in April 1979, but clearly rests on the assumption that the catching efficiencies in the two periods were equivalent. Nevertheless, as table 5 shows, the two types of estimate were very similar for both males and females, and in each case they fell between the maxima and minima previously computed. Thus, the most appropriate density values for April 1979 appear to be the means of these two estimates (males $0.074/m^2$, females $0.0272/m^2$), leading to numbers within the panmictic circle of males 6807, females 2176.

TABLE 5
Capture-recapture data and estimates, April 1979

	4-7 April 1979	
	♂♂	♀♀
Final catch		
Unmarked	131	50
Day 1—marked (Nos. released in brackets)	7(83)	3(28)
Day 2—marked (Nos. released in brackets)	5(71)	3(30)
Day 3—marked (Nos. released in brackets)	5(31)	1(14)
Population size	1531	522
Population density (m^{-2})	0.080	0.027
August density (m^{-2}) $\times \frac{\text{Nos. caught April 1979}}{\text{Nos. caught August 1978}}$	0.068	0.026

Having derived these estimates of N_e for August 1978 and April 1979, it is necessary to derive (interpolated) estimates of density for each generation during the year. The ecology of the Mount Parnes population is not sufficiently well known to allow its breeding-pattern to be described with certainty. But if, in the light of the climate on Mount Parnes and the limited data on reproductive condition (table 2), we draw an analogy with Begon's (1976*b*) results for an English population of *D. subobscura*, the following pattern is suggested: that there are breeding peaks in late-March/early-April, early-May, early-June, early-July, early-August, early-September and early-October. Thus, we have data on the first and fifth of these peaks.

As previously mentioned, extensive collection data from Mount Parnes suggests that the early-August density is typical of the six "summer" generations. To derive our minimum estimate of N_e , however, we will assume that there is a consistent rise from the first to the fifth generation, followed by a plateau, and then the expected drastic decline over the winter months. Such an assumption leads to the first three columns of figures in table 6. At the bottom of the third column, following Wright (1969), the overall effective size has been obtained by taking the harmonic mean.

TABLE 6

Minimum estimates of effective population numbers for the Mt. Parnes D. subobscura population

	♂♂	♀♀	Total	Variance effective number N_e ($s_k^2/\bar{k} = 14.38$)
March-April	6,807	2,176	6,596	858
May	22,354	24,799	47,026	6,115
June	37,901	47,422	84,261	10,957
July	53,448	70,045	121,262	15,769
August	68,994	92,668	158,195	20,572
September	68,994	92,668	158,195	20,572
October	68,994	92,668	158,195	20,572
Harmonic mean			33,027	4,296

Such calculations assume, however, that the distribution of family sizes amongst parents is random, or, in other words, that the variance-to-mean ratio is unity. This assumption is certainly false. It is, however, impossible to obtain a correct variance-to-mean ratio from the natural population itself. Begon (1977) chose to use a value of 14.38, obtained from newly-established laboratory strains pairmated under optimal conditions, where the mean family size (of adults surviving to reproduce themselves) was approximately 88. He argued that, since differentials in many fitness parameters were being ignored under these conditions, this estimate was likely to be conservative. Crow and Kimura (1970) on the other hand, following Crow and Morton (1955), have argued that variance-to-mean ratios obtained when the family size is large should be reduced by assuming random mortality, before being applied to populations in which the growth-rate is not so high. Overall, therefore, the situation is essentially unresolved. In the present case we shall use the value of 14.38 for all generations in our minimum estimate (column 4 of table 6), and the modification suggested by Crow and Kimura in our "best" estimate.

We ought, finally, to consider the pattern of mating, but, since we have no information on this, we must ignore it. Nevertheless, we feel confident in asserting that, in view of our consistent tendency to underestimate N_e , the effective size of the *D. subobscura* population on Mount Parnes, estimated ecologically, is at least 4296 (table 6).

To obtain our "best" estimate of N_e we contend that the appropriate T -value for *D. subobscura* is about 20 days; that, in estimating density, the grid should only be extended 15 m in each direction; that all summer populations are as dense as the one examined in August 1978; and that the variance-to-mean ratio should be modified as suggested by Crow and Kimura (1970). As table 7 shows, an effective size of 92,604 is suggested. If additional allowance were made for migration between panmictic circles, an increase of at least one order of magnitude would be appropriate.

TABLE 7

"Best" estimate of effective population number for the Mt. Parnes *D. subobscura* population

	♂♂	♀♀	Total	N_e
March-April	19,668	6,288	19,059	18,939
May	198,798	267,803	456,396	98,341
June	198,798	267,803	456,396	396,161
July	198,798	267,803	456,396	396,161
August	198,798	267,803	456,396	396,161
September	198,798	267,803	456,396	396,161
October	198,798	267,803	456,396	396,161
Harmonic mean				92,604

5. DISCUSSION

It is beyond dispute that genetic drift can only effect measurable changes in gene-frequency in small populations, but the distinction between large and small populations is not a clear one. It is safe to assert, however, that an effective population number of 4000 is indistinguishable from infinity as far as these effects of drift are concerned (Wright, 1943). Thus, in the present

case, two of our methods—the ecological method and the lethal allelism method—suggest that N_e for the Mount Parnes *D. subobscura* population is effectively infinite. The results of the temporal method, on the other hand, are rather more equivocal: the estimate for the second time period is infinity, and, overall, sixteen out of the eighteen “individual estimates” seem to agree with this; but the estimate for the first time-period itself is only 268. The preceding sections make it clear, however, that whereas in the ecological method we have been at pains to produce a *minimum* estimate, and the lethal allelism method requires only that the mutation-rate to lethal alleles be equal at different loci, the temporal method rests heavily on the assumption that genic variation is selectively neutral. We are confident, therefore, that the results of the ecological and lethal allelism methods (confirmed in 16 out of 18 cases by the temporal method) are qualitatively correct: the effective size of the *D. subobscura* population on Mount Parnes is at least 4000, and effectively infinite.

Thus, having fulfilled our second aim by outlining these three estimation methods, we can turn to our first aim and consider this estimate in relation to previous work. The major difficulty in doing so is that all estimates are to some extent imperfect, and it is generally impossible to completely disentangle real differences from differences attributable to methodological inadequacies.

Studies of plants—based on seed-dispersal where pollination is by insects—have yielded neighbourhood numbers of 4–5 in *Lithospermum carolinense* (Kerster and Levin, 1968) and 75–290 in *Phlox pilosa* (Levin and Kerster, 1968). Wilson (1975) states that the effective deme size of many vertebrates is between 10–100; Kerster (1964), considering dispersal and the variation in offspring numbers, found that N_e in the lizard *Sceloporus olivaceus* was 225–270, while Merrell (1968) estimated the effective number in the frog, *Rana pipens*, to be 46–112, by counting the number of egg-masses laid in each pond. Greenwood (1975), in an extensive study of the snail *Cepaea nemoralis* which nevertheless ignored inter-population migrants, estimated N_e to be between approximately 200 and 6000. Dobzhansky and Wright (1943), using the lethal allelism method, estimated the effective size of a *Drosophila pseudoobscura* population as 500–1000; while Crumpacker and Williams (1973), considering numbers of *D. pseudoobscura* in a panmictic circle during a single (summer) generation, obtained estimates of between approximately 3000 and 6500. Begon (1977) working with *D. subobscura* and ignoring mating pattern and interpopulation migrants, estimated N_e for an English population restricted to a small wood to be around 400. And finally, Lande (1979), basing his calculations on the rates of chromosomal rearrangements, estimated N_e to be 30–200 in mammals, 40–300 in lower vertebrates and 200–800 in dipterans like *Drosophila*.

So, irrespective of the difficulties in interpretation, it is clear that our present estimate is large in comparison with those of previous workers. This is true of comparisons across diverse taxa, but also within the *obscura*-group *Drosophila* since Crumpacker and Williams' (1973) figures apply to the yearly peak of abundance. It is interesting to note, moreover, that Jones and Parkin (1977), using information on inter-“population” migrants, also conclude that *D. pseudoobscura*'s effective population size must be extremely large.

The present estimate has been derived independently from a variety of methods, and, in the case of the ecological method, has been purposely underestimated. We therefore feel that the present estimate, in its general ("certainly exceeding 4000") rather than its specific ("4296" or "92,604") form, is worthy of considerably more confidence than are those of previous workers who have relied on very partial methods of estimation. It is quite clear, in other words, that the effective size of a natural population can be extremely large. It should be noted in this respect, however, that data on chromosomal polymorphism indicate that *D. subobscura* populations from Greece lie in an ecologically central area of the geographic distribution (Krimbas and Loukas, 1980); and also that the frequency of lethal chromosomes is much higher in Greece than in other populations studied (England, Vienna, Norway)—another indication of ecological centrality (Loukas *et al.*, 1980). Central populations are generally regarded as being of larger size than marginal ones.

In considering our third aim—throwing light on the validity of the three methods—it would be wrong to claim that the assumptions implicitly or explicitly made by the lethal allelism and ecological methods have been proved correct. It is possible to assert only that these methods have proved adequate in the present case. It is obvious, however, that the crucial assumption of the temporal method—that the genic variation is selectively neutral—appears to be unsupported by the data. Specifically, there seems to have been directional selection at the *Est-5* and *Aph* loci, and this is undoubtedly worthy of further investigation. By comparing the temporal with the other methods, in other words, we have been able to pin-point these potentially important selection events. Regarding the other loci, however, it is impossible to distinguish between stabilizing selection and neutrality.

As Lewontin (1974) has pointed out, the evidence "in favour" of neutrality is often actually only *compatible* with the neutrality hypothesis. In the present case, the data from these eight loci are indeed compatible with neutrality—but equally compatible with stabilizing (or, for that matter, weak directional) selection.

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