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# Are geographic populations equivalent to genetic populations in biennial species? A study using *Verbascum virgatum* (Scrophulariaceae)

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SUMMARY

Geographic populations are not necessarily equivalent to genetic populations. Electrophoretic isozyme analysis shows that in the biennial, *Verbascum virgatum*, not only is spatial distribution a factor creating isolated populations, but time also is an isolating mechanism. Thus, in the biennial situation there is a possibility that selection can be rejected as the probable cause of variation, much as can random drift in cases involving spatial distributions. The magnitude of difference in frequency of alleles between the odd and even year colonies studied was too large to be explained by random drift in populations of the size and short duration of those we observed. Similarly, it is unlikely that random fluctuations in selection intensity on one age class would produce a difference as large as that observed. It is possible, however, that variation was introduced (mutation or founder) when the population was much smaller and that the difference was trapped at a relatively high level when the population rapidly increased in size. Simulations and algebraic theory do not refute this idea. They also show that colony differentiation can occur with migration rates considerably greater than previously predicted.

## 1. INTRODUCTION

Biennial plants need two seasons from the time of seed germination to seed formation. If all seeds set one year germinate in the next growing season, then all individuals will complete the life cycle in two years. These plants will be reproductively out of phase with those derived from seed set a year later. It is possible, therefore, for two genetically and temporally isolated colonies to occur in the same geographic population. Migration between temporally isolated genetic colonies of a geographic population occurs when individuals complete the life cycle in an odd number of years, as when individuals become perennial (i.e. reproduce two or more years in a row), or mature in one or three years rather

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than two. Migration can also occur if seeds germinate after remaining dormant for one or more years.

In nature, random drift of neutral mutations can produce allele frequencies which are similar to those which result from selection (Kimura & Maruyama, 1971). However, when selection is uniform in a biennial species at a given geographic site, the difference in allele frequencies between odd and even year colonies must be a function of random drift or founder effects. Even yearly fluctuations in environmental selection for an allele should not produce allele frequency differentiation between colonies, since representatives from both odd and even year plants are always present. Only if selection is limited to one age class, and fluctuates yearly, may differentiation be produced. In this last situation, the expected degree of difference can be estimated, and in some cases, selection can be rejected as the probable cause of the differences. The biennial organism, therefore, is a powerful tool for studying drift and migration.

There have been only a few evolutionary studies where the biennial (tri-ennial, etc.) life history has been used as a tool to control for selection. The best, perhaps, is the study of the pacific pink salmon (*Oncorhynchus gorbuscha*) by Aspinwall (1974). He studied both temporal and spatial heterogeneity of salmon. It was found that genetic variation which might normally be used as evidence for selection could be better accounted for by the neutral mutation-random drift hypothesis when odd and even year colonies were examined.

Angiosperms are an untapped source of biennials for evolutionary study of migration and random drift. In *A California Flora* alone (Munz & Keck, 1970), we have found more than 160 biennial species listed. Although not previously used as a control for selection, angiosperm biennials have been extensively studied for their interesting life histories (Cleland, 1949, 1950, 1962, 1964, 1972; Munz, 1949, 1965; Levy & Levin, 1975; Levy, Steiner & Levin, 1975; Schaffer & Gadgil, 1975; Werner, 1976; Hart, 1977; and Platt & Weiss, 1977).

*Verbascum virgatum* is a native of Eurasia, and a biennial member of the family Scrophulariaceae (Munz & Keck, 1970). The plant morphology is typical of biennial angiosperms: a rosette is formed the first year and bolting occurs the second. The second year stem is 40 to 120 cm tall with approximately 30 flowers. The flowers are slightly irregular and yellow in colour. Seeds are less than 1 mm in diameter with no obvious means of dispersal.

We have studied colonies of two populations (as defined by Kimura, 1953) of *Verbascum virgatum*. These populations are disjunct and isolated from other populations of the species. Acrylamide gel electrophoresis was used to compare enzyme proteins of odd and even year genetic populations at each of the two geographic sites.

The finite circular stepping-stone model of population structure (Maruyama, 1970; Kimura & Maruyama, 1971) with only two colonies fits the *V. virgatum* populations at the sites used. Each population has two colonies, one reproducing in even number years and the other in odd number years. Progeny of individuals which reproduce in one or three years are migrants, as are progeny of an individual

which has reproduced two years in a row, or seeds which remain dormant one season. Since migration is from 'neighbouring' colonies and because there are no terminal colonies in the sense of the linear stepping-stone model (Kimura, 1953; Kimura & Weiss, 1964; and Maruyama, 1971), the biennial situation fits the circular stepping-stone model where only two colonies exist. That model predicts that the rate of decline of heterozygosity is $m\pi^2/(2n)^2$ when $2Nm < 2n/10$, and is approximately $1/(4nN)$ when the inequality is reversed; where $2n$ = the number of colonies, $N$ = the number of diploid individuals per colony, and $m$ = the migration rate. Also predicted from simulations was that 'marked colony differentiation' for the circular stepping-stone model should occur if $2Nm < 2n/\pi^2$.

We have measured isozyme frequencies electrophoretically in temporal colonies of two geographic populations of *Verbascum virgatum*. Then we derived the algebraic expression for the mean and variance of the expected difference in allele frequencies between temporal colonies in the next generation, given the allele frequencies of the two temporal colonies in the present generation. The probability of a decrease in the difference in allele frequency between odd and even year colonies in the next generation, given present allele frequencies, is also derived and the results are graphed for selected migration rates and population sizes. Finally, computer simulations were performed to confirm the algebraic results, to test whether the model can produce the results we observed in the field, to test whether fluctuations in selection intensity on only one stage of the life cycle could have produced the difference observed, and for the two colony case, to test the theoretical point predicted by Kimura & Maruyama (1971) below which 'marked colony differentiation' should occur.

## 2. MATERIALS AND METHODS

*Verbascum virgatum* individuals from two geographic populations on the campus of Occidental College (Los Angeles, California, U.S.A.) were analysed for genetic differences by acrylamide electrophoresis of proteins. The species was chosen for study because it has been reported to be a 'true' biennial and is of European origin. Since *Verbascum* has been introduced from Europe, the maximum age of local populations can be estimated. The pueblo of Los Angeles was not founded until 1781, so the populations studied are unlikely to be more than about 100 generations old (200 years), and could well be of more recent origin.

The Occidental College campus is located on a granitic hill in the middle of a valley approximately 5 km wide. There are populations of *V. virgatum* on two west facing hills separated by approximately 120 m. Seeds have no visible means of dispersal, and were never observed to fall more than 1 m from the parental plant. The populations studied appear to be isolated from other populations of the species and possibly from one another. Apparently isolated populations of *V. virgatum* were chosen for study because the stepping-stone model has not yet been developed for a biennial species when spatial (geographic) as well as

temporal colonies are linked through migration. What would be a linear stepping-stone model for an annual species, for example, might be likened to a ladder for a biennial species with spatial migration (Fig. 1). If migration rates are high neither spatial nor temporal genetic differentiation would be observed.

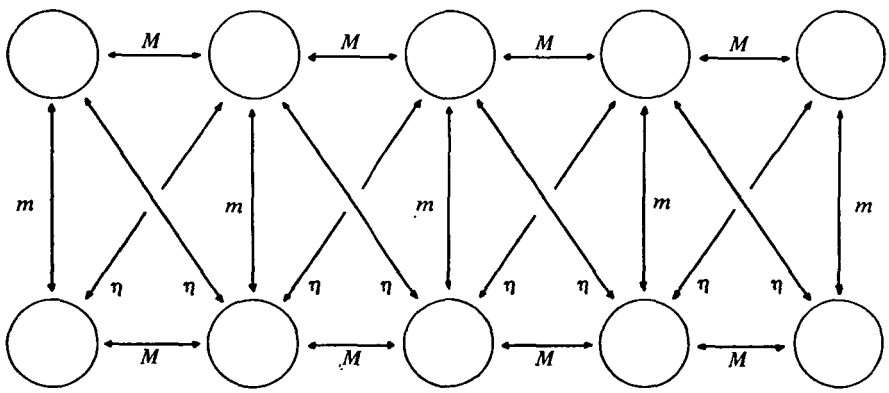


Fig. 1. Representation of a biennial species which fits a finite linear stepping-stone model. Bottom row of circles are odd year geographic colonies, and the top row are their respective even year geographic colonies. $M$ is migration between neighbouring geographic colonies of the same year class, $m$ is migration between odd and even year colonies of a geographic colony, and $\eta$ is migration between opposite year colonies of neighbouring geographic colonies.

Sizes of the populations were estimated, and samples were taken in a single year to avoid the complication of drift between years. Twenty-five rosette and 25 flowering plants were tagged from the eastern geographic population, and 20 rosette and 20 flowering plants were tagged from the western populations. Basal leaves were removed as needed for electrophoresis. Since these populations are close to laboratory facilities fresh material was always used immediately upon harvest. Slab acrylamide electrophoresis was used to separate isozymes. Samples were run with 8% main gel/5% stacking gel, and 4% main gel/3% stacking gel. The buffers were borate-NaOH pH 8·0 electrode/pH 8·5 gel (Shaw & Prasad, 1970: II 0·3 M-borate), and tris-borate pH 7·5 electrode/pH 7·5 gel (Shaw & Prasad, 1970: XI 0·0546 M-tris 0·0245 M-borate). Equal leaf surface areas of each plant were ground in the electrode buffer to be used in the run. Samples were run in duplicate, with those from the two year classes loaded randomly into gel slots, and they were read 'blind'. A constant 120 V was applied to the gels. The apparatus was run until a blue food die marker reached the end of the gel. Haemoglobin and lactalbumin were used as internal standards. The internal standards were in three slots for each gel (centre and both ends). Since lactalbumin is not visible, gel strips containing the internal standards were cut from the gel prior to staining for an enzyme, and stained with a general protein stain (Johnson, 1975: TCA-coomassie blue). Enzyme loci studied were acid phosphatase, alkaline phosphatase, leucine aminopeptidase (LAP), peroxidase, glutamate-oxaloacetate transaminase (GOT), and 'general protein'. Stain formulas are from Shaw & Prasad

(1970), except for the general protein stain. Gels were fixed in 10% acetic acid except for those stained for peroxidase. Peroxidase stained gels were fixed in 50% glycerine. Electrophoretic bands are numbered sequentially according to enzyme mobility on 8%-pH 8·5 gels. The only exception is the phenotype denoted 'null' which had no staining. Results are expressed as electrophoretic phenotypes (genotypes), rather than alleles, since, if all allele frequencies were calculated, there would be total loss of degrees of freedom and statistical testability. The problem is the same as that of the ABO blood types, as discussed by Spiess (1977). Even so, use of 'genotypes' rather than 'allele' frequencies is statistically conservative in that it minimizes sample size. We used the $\chi^2$ and a Generalized Exact Test (Wells & King, 1980) to assess significance of differences between electrophoretic phenotype frequencies in odd and even year colonies from each geographic location, and between geographic populations.

Next we considered theoretical models. First algebraic results were studied, then computer simulations. Derived were the mean and variance of the expected difference in allele frequency between the next generation's temporal colonies due to drift, given allele frequencies in the present generation of a single geographic population for a biennial organism where binomial sampling is assumed. Using the derived mean and variance, the distribution of expected difference in allele frequency between the next generation's temporal colonies of a geographic population can be approximated by a normal distribution when $n > np \pm 3(npq)\frac{1}{2} > 0$ and $n > nr \pm 3(nrs)\frac{1}{2} > 0$; where $p$ = even year allele frequency ($q = 1-p$), $r$ = odd year allele frequency ($s = 1-r$), and $n$ = two times the number of diploid individuals per colony. Using the normal approximation we have calculated and graphed the probability that the difference in allele frequency will decrease between the next generation's odd and even year populations.

Monte Carlo simulations were performed on a TRS-80 computer. Four types of simulations were performed. Our *V. virgatum* data and algebraic predictions were compared to the results of each set of simulations.

The first set of computer simulations tested the effect of random fluctuations in selection intensity which acted only on one stage of the life history (rosette or flowering). Trials were initiated with identical allele frequencies in each colony. Results were recorded as the mean and variance of the absolute difference observed in allele frequency between odd and even year colonies. The remaining simulations tested the effect of random phenomena.

The second set of trials was initiated with a single hypothetical mutant in one of the two colonies. Each trial was run until extinction of an allele occurred or for 110 generations, whichever came first. Simulation results were recorded as time to extinction, and as the mean and variance of the absolute difference observed in allele frequency between odd and even year colonies. In the third set of trials the initial allele frequencies were 0 and 1 for the respective colonies. Simulations were run for 110 generations to determine the level at which decrease in difference between temporal colonies becomes very slow, and whether the rate of decrease becomes very slow within the age limit of the populations. For each

population size and migration rate 50 trials are lumped and the results expressed as a linear regression of allele frequency for the last 10 of 110 generations (where decrease is slowest). Lastly, computer simulations were run where a single hypothetical mutation was introduced into a very small population ($N = 5$ diploid individuals) which then grew rapidly to a maximum size ($N = 640$ diploid individuals) at which it remained for the succeeding generations. Results of these trials are expressed in the same way as those from the first set of trials.

Finally, the predicted rate of decline of heterozygosity (Maruyama, 1970), and the predicted point below which marked colony differentiation should occur (Kimura & Maruyama, 1971) for the two colony stepping-stone model were calculated and compared to results of the simulations and to the field data.

## 3. RESULTS AND DISCUSSION

The size of each geographic population was estimated by counting the number of rosettes and the number of flowering individuals. The eastern geographic population had 360 flowering individuals and 685 rosette individuals. The flowering generation of the western population was smaller than that of the eastern population: 711 rosette individuals and only 98 flowering individuals.

Peroxidase and LAP were the only enzymes which showed variation in the populations studied. Table 1 lists the electrophoretic data of the enzymes which showed variation. The $\chi^2$ test (see Yarnold, 1970; and Larntz, 1978, for the relaxed requirements of expected cell frequency), and the Generalized Exact Test (Wells & King, 1980), which calculates the exact probability of obtaining a $Q$ value (a $\chi^2$ value) as large or larger than that actually observed for a given table and has no minimum expected cell frequency, were used to test for differences in genotype frequencies between colonies and between populations. LAP phenotype frequencies of the eastern population differ significantly between odd and even year colonies ($p = 0{\cdot}00332$). The western geographic population, however, showed no significant difference in LAP electrophoretic phenotype frequencies ($p = 0{\cdot}487$), nor did peroxidase phenotypes ($p = 1{\cdot}0$) in either geographic population. The peroxidase locus 'A' which has alleles in approximately equal frequency in odd and even year colonies of the geographic populations, has significantly different frequencies in the two geographic populations (G.E.T. $p = 0{\cdot}0000754$, $\chi^2\ p < 0{\cdot}01$). Both rosette and flowering LAP phenotype frequencies also differ significantly between geographic populations (G.E.T. $p = 0{\cdot}000686$ and $p = 0{\cdot}00365$; for both, $\chi^2\ p < 0{\cdot}01$). Thus, the electrophoretic data support our assumption that the geographic populations studied are isolated from one another. More interesting than geographic isolation, however, is the fact that the electrophoretic data show the odd and even year temporal colonies of a geographic population are genetically isolated. If isolation were not pronounced, differentiation would not be observed.

It is of interest next to ask: What conditions (e.g. migration, selection, population size) might lead to the degree of genetic differentiation observed between odd and even year colonies of a geographic population? Since model results are

Table 1. *Contingency tables of electrophoretic phenotypes by colony*

| LAP phenotype | Eastern geographic population: Rosette | Eastern geographic population: Flowering |
|---|---|---|
| Band 2 | 15 | 15 |
| Band 1 | 0 | 7 |
| Null | 10 | 3 |

$\chi^2 = 10{\cdot}77$, D.F. $= 2$, $P < 0{\cdot}01$. Generalized Exact Test $P = 0{\cdot}00332$.

| LAP phenotype | Western geographic population: Rosette | Western geographic population: Flowering |
|---|---|---|
| Band 2 and 3 | 2 | 0 |
| Band 2 | 18 | 20 |

Expected cell sized too small for $\chi^2$. Generalized Exact Test $P = 0{\cdot}487$.

| Peroxidase A phenotype | Eastern geographic population: Rosette | Eastern geographic population: Flowering |
|---|---|---|
| Band 1 | 18 | 17 |
| Null | 7 | 8 |

$\chi^2 = 0{\cdot}0952$, D.F. $= 1$, $0{\cdot}7 < P < 0{\cdot}9$. Generalized Exact Test $P = 1{\cdot}0$.

| Peroxidase A phenotype | Western geographic population: Rosette | Western geographic population: Flowering |
|---|---|---|
| Band 1 | 20 | 20 |

No electrophoretic differences.

| Peroxidase B phenotype | Eastern geographic population: Rosette | Eastern geographic population: Flowering |
|---|---|---|
| Band 1 | 1 | 4 |
| Null | 24 | 21 |

$\chi^2 = 2$, D.F. $= 1$, $0{\cdot}1 < P < 0{\cdot}2$. Generalized Exact Test $P = 0{\cdot}348$.

| Peroxidase B phenotype | Western geographic population: Rosette | Western geographic population: Flowering |
|---|---|---|
| Null | 20 | 20 |

No electrophoretic differences.

in terms of allele frequencies the first step is to estimate the difference in an allele's frequency between colonies. Although it is a difficult task to estimate all allele frequencies, it is easy to estimate the null allele frequency. A good estimate of the null allele frequency is simply the square root of the observed null phenotype frequency (rosette = 0·632, flowering = 0·346). The difference in null allele frequency then is $\Delta = 0{\cdot}286$ and, according to the method of Bailey (1975), the 95% confidence interval is 0·0983 to 0·474.

The next step is to calculate the mean and variance of the difference in allele frequency between odd and even year colonies of a geographic population in the next generation, given only the allele frequency in the colonies in the present generation in the theoretical model. Assume equal even and odd year colony size, stable population size, and let $N$ be the number of diploid individuals per colony. Let $p$ = the frequency of allele 'A' in the present generation of the even year colony ($q = 1-p$), and $r$ = the frequency of allele 'A' in the present generation of the odd year colony ($s = 1-r$). Finally, let $m$ be the number of migrants leaving a colony and reaching the other colony per generation, and let $\Delta = p-r$. The mean of $\Delta$ expected for the next generation, $E(\Delta)$, then is $(1-2m/N)\Delta$ where, $m$ = the number of migrant individuals, and $N$ = the number of individuals per colony. The variance of $\Delta$ expected for the next

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generation, Var(Δ), has been derived by first calculating $E(\Delta^2)$ and then using the fact that $\mathrm{Var}(\Delta) = E(\Delta^2) - E(\Delta)^2 . E(\Delta^2) = (pq+rs)/2N + [(1-2m)/N]^2\Delta^2$ so $\mathrm{Var}(\Delta) = (pq+rs)/2N$.

The distribution of Δ expected in the next generation is approximated by a normal distribution based upon the derived mean and variance. A normal approximation was used to calculate the probability that $|\Delta|$ in the next generation will be less than $|\Delta|$ in the present generation. The results are graphed for $N = 500$, $m = 1$ and $m = 5$ in Fig. 2 (graphs for $N = 5000$, $m = 1$ and 50; and for $N = 50$,

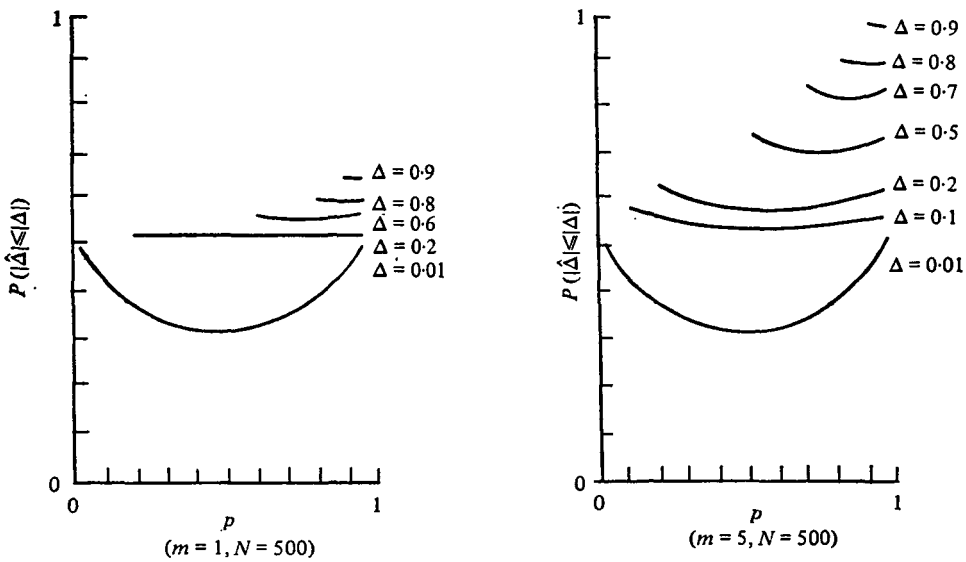


Fig. 2. Probability that $|\hat{\Delta}| \leqslant |\Delta|$ given only the allele frequencies in the present generation. $\hat{\Delta} = \Delta$ in the next generation, $p$ is the frequency of the allele under study in the even year colony, and the allele frequency in the odd year colony is given by Δ (i.e. $r = \Delta - p$).

$m = 1$ and 5 are available upon request). Let $\hat{\Delta}$ denote Δ of the next generation. Fig. 2 shows that for a given $N$ and $m$ there is a Δ of the present generation for which the probability $P(|\hat{\Delta}| \leqslant |\Delta|)$ will be less than the probability $P(|\hat{\Delta}| > |\Delta|)$. Therefore, except for random fluctuations, Δ should remain relatively constant once it reaches the point where $P(|\hat{\Delta}| \leqslant |\Delta|) = P(|\hat{\Delta}| > |\Delta|)$.

Through computer simulations, each of 100 generations, we tested the possibility that random fluctuations in selection intensity confined to one life stage (rosette or flowering) could produce a differentiation as large as that observed. Results obtained for a codominance allele system are shown in Table 2. Other modes of inheritance and breeding (self-fertilization) gave similar results (available upon request from the authors). The data do not support the contention that selection could produce the differentiation we observed in populations of the age and structure of those studied. The 95% confidence interval of the difference observed in allele frequency of the studied population in no case overlapped the

Table 2. *Computer simulation of a two colony stepping-stone model*

(Selection intensity fluctuates randomly on a yearly basis and is normally distributed. Selection acts only on the rosette stage.)

| Selection coefficient | | Codominance* | |
|---|---|---|---|
| Mean | S.D. | Mean for 50 simulations of the mean difference in allele frequency for 100 generations | S.E. of the mean absolute difference ($N = 50$) |
| 0·5 | 0·25 | 0·0336 | 0·0218 |
| 0·5 | 0·05 | $7{\cdot}21.10^{-3}$ | $4{\cdot}52.10^{-3}$ |
| 0·25 | 0·125 | 0·0351 | 0·0185 |
| 0·25 | 0·025 | $7{\cdot}88.10^{-3}$ | $5{\cdot}46.10^{-3}$ |
| 0·1 | 0·05 | 0·0295 | 0·0167 |
| 0·1 | 0·01 | $6{\cdot}70.10^{-3}$ | $3{\cdot}76.10^{-3}$ |
| 0·05 | 0·025 | $4{\cdot}83.10^{-3}$ | $3{\cdot}24.10^{-3}$ |
| 0·05 | 0·005 | $1{\cdot}16.10^{-3}$ | $7{\cdot}50.10^{-4}$ |

* Selection against $AA = s$, $Aa = s/2$, $aa = 0$; initial frequency of $A = 0{\cdot}99$.

interval defined by $\pm 2$ standard errors of the simulation means. This includes simulations with a very large standard deviation compared to the mean selection coefficient (if the standard deviation were any larger than $\frac{1}{2}$ the mean selection coefficient the variation could no longer be considered to be normally distributed) and for different modes of gene expression.

In fact, even high self-fertilization levels with clonal selection for groups of linked genes, as has been suggested to occur in barley (Clegg, Allard & Kahler, 1972; Kahler, Clegg & Allard, 1975), would not account for the differences we observed. Neither the 95% confidence interval for the null allele (0·117, 0·443 assuming selfing) or band 1 allele (0·160, 0·400 assuming selfing) overlaps the interval defined by $\pm 2$ standard errors of the simulation means. This included cases where the selection coefficient is probably higher than would be expected even when selection occurs for a group of linked genes ($s = 0{\cdot}5$, $s = 0{\cdot}25$).

Computer simulations where single neutral mutations are introduced into the population were run for population sizes from $N = 50$ to $N = 10{,}000$. Two types of value are of interest. They are the value of simulations with extreme mean difference in allele frequency ($\mu_{|\Delta|} + 2\sigma_{\mu|\Delta|}$), and the extreme values of simulations with extreme mean difference in allele frequency ($\mu_{|\Delta|} + 2\sigma_{\mu|\Delta|} + 2\sigma_{|\Delta|}$). Only simulations with parameters $N = 50$, $m = 0$ had extreme mean differences in allele frequency (0·1204) within the 95% confidence interval of the observed difference in null LAP allele frequency. Furthermore, only simulations with parameter $m = 0$ and $N = 50$ or $N = 100$ had extreme values of trials with extreme mean difference (0·1673 and 0·1110) within the 95% confidence interval of the observed difference in null LAP allele frequency. It is apparent that even with zero migration and a population size substantially less than our estimate (360 individuals), the $\Delta$ we observed would be an extremely rare occurrence in

a population with a maximum age of that known for *Verbascum virgatum* in the Los Angeles basin.

The results of computer simulations with neutral alleles where initial frequencies were $p = 1$ and $r = 0$ were next considered. The results were expressed as regressions of the last 10 of 110 generations where 50 values of $Y$ exist for every value of $X$, and showed that the decrease in $|\Delta|$ is very slow after 100 generations and that $|\Delta|$ is substantially greater than in simulations where single mutations were introduced. For example, the maximum slope was $-0{\cdot}005$ ($N = 50, m = 1$). Simulations with larger population sizes had a slower decrease in the difference between classes when migration numbers were the same. From the results it is obvious that, if a population initially has a large $\Delta$, a greater difference in allele frequency than expected can be maintained for much longer than the existence of the populations we studied. The probabilities we calculated ($P(|\hat{\Delta}| \leqslant |\Delta|)$ in Fig. 2) agree with the conclusions from the simulations.

Table 3. *Computer simulation of a two colony stepping-stone model with one initial mutation and changing population size*

| Diploid individuals | | Mean of $\|\Delta\| = \mu\|\Delta\|$ | |
|---|---|---|---|
| $N$** | $m$ | Mean* of $\mu_{\|\Delta\|}$ | S.D.* of $\mu_{\|\Delta\|}$ |
| 640 | 3 | 0·0688 | 0·0860 |
| | 10 | 0·0398 | 0·0388 |
| 1280 | 3 | 0·0779 | 0·0960 |
| | 10 | 0·0565 | 0·0644 |

* $n$ of 200 trials.

** $N$ at time zero = 5, remained at that size for one generation, and then doubled in size every generation until $N$ given was reached. Migration was probabilistic when $N$ was increasing ($p = m/N$) after which $m$ migrants/generation occurred in each colony.

If the populations began as a few individuals and rose rapidly to their present level, and if either single mutations occurred when the populations were small or genetic diversity existed in founding individuals, then the $\Delta$ we observed is possible (Table 3). As an example, the difference between a mutation introduced into a small population ($N = 5$ individuals) which then increased rapidly to 640 individuals, and a population in which a mutation occurs but the population size is already 640 individuals, is shown in Fig. 3. Nei, Maruyama & Chakraborth (1975) have shown that if population size increased rapidly after going through a bottleneck, reduction in heterozygosity would be small. However, loss in the average number of alleles per locus would be considerable. Both the simulation and the observations made on the natural populations agree with Nei's predictions.

The asymptotic rate of decline of heterozygosity predicted by Maruyama (1970) for populations with the parameters we studied is approximately 0·005. The simulation, probabilities graphed, and observed field data are consistent with this prediction. Additionally, our simulations predict that, even with considerable migration, the difference in allele frequency between colonies (temporal in our study) may persist at levels much greater than Kimura & Maruyama (1971) expected.

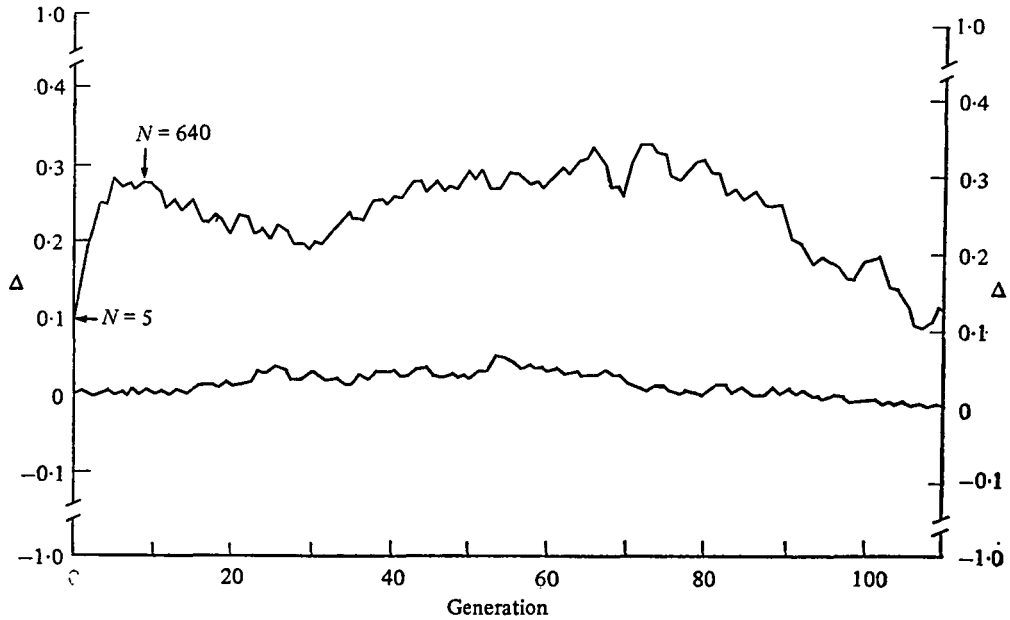


Fig. 3. Example of two simulations. Top line displays an example in which a single mutation occurred at time zero in a population of size $N = 5$. The population remained at $N = 5$ for one generation, and then doubled in size every generation until $N = 640$, at which size it remained for the remaining generations. Migration when $N = 640$ was three individuals each direction per generation. When $N < 640$, migration was calculated using binomial sampling with a random number generator ($p = 0{\cdot}00469$ of a given individual being a migrant). Bottom line displays an example in which a single mutation occurred at time zero in a population of constant size, $N = 640$, and with constant migration of three individuals each direction per generation.

The migration rate predicted by the theoretical work of Kimura & Maruyama (1971), below which marked colony differentiation of allele frequency should occur for a circular stepping-stone model of two colonies with the population size we observed, is approximately 0·1 individual per generation in each direction. It is apparent from the simulations where single mutations occur in a population of fixed size that the marked colony differentiation illustrated by Kimura & Maruyama is very unlikely even when the migration rate is zero. The life span of our *Verbascum* colonies, and their corresponding simulations, is too short to realize the Kimura-Maruyama predictions. We suggest that the colony differentiation we observed resulted when a very small population, with variation, rapidly expanded to a much larger size. Since many North American plant species have an Old World origin, and many others briefly inhabit temporarily disturbed localities, the process we have described may aid in the interpretation of other field observations.

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