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POPULATION STRUCTURE AND GENE FREQUENCY ANALYSIS OF SIBLING SPECIES OF *LETHE*

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ABSTRACT. Two recently described sibling species of *Lethe* (*L. appalachia* and *L. eurydice*) exist sympatrically at McLean Bogs Reserve, Tompkins County, New York. We examined the population structure and electrophoretic variation of these two species and found they are substantially different. *L. appalachia* exhibits high vagility in its preferred woodland habitat; delineation of spatial population units was not feasible. *L. eurydice* is philopatric and local in wet meadows; demographic data are easily obtained for this sedentary species. Interspecific comparisons of eight enzyme-synthesizing loci revealed significant differences in allele frequencies at five loci, providing further evidence of two separate gene pools. On the basis of these eight loci, the calculated genetic distance between the two species is 0.145, well within the range of values previously reported for other sibling pairs.

The existence of a pair of sibling species in the genus *Lethe* in eastern North America was proposed several years ago (Cardé, Shapiro and Clench, 1970; Shapiro and Cardé, 1970). It had long been recognized (Field, 1936; Chermock, 1947) that at least two morphologically distinct forms of *Lethe eurydice* occurred in the eastern United States. It was also noted that most reports of this species from northern localities were from open, wet sedge meadows. In the southern portion of the range, however, all reported individuals were observed in deep woods or bush swamp. Chermock had placed all specimens south of Pennsylvania in the subspecies *L. e. appalachia.* Cardé *et al.* recognized that the two forms were, in fact, widely sympatric, habitat-isolated sibling species. They demonstrated that a variety of subtle but consistent morphological characters of adult genitalia and wing patterns as well as larval markings could be used to distinguish the specimens flying in closed woods from those appearing in open meadows. *Lethe eurydice* is the species characteristic of open meadows. Its range extends from southern Alberta and eastern North Dakota to Nova Scotia, and southward to Delware, Pennsylvania and Ohio. *Lethe appalachia* is restricted to shady, closed canopy woods and bush swamp within sight of water. It is found in the Appalachian ranges from Georgia to Maine, and in the northern part of the range extends westward to Michigan.

A study was undertaken at McLean Bogs Reserve, Tompkins Co., New York to test the accuracy of Cardé and Shapiro's analysis of the *Lethe* situation. A site was chosen for sampling where putative *L. eurydice* habitat was available directly adjacent to *L. appalachia* habitat. A mark-recapture program was begun in the *L. eurydice* habitat (a very well-

marked depression with saturated soil and numerous *Carex* species, surrounded by drier, well-grazed pasture) in order to estimate the density of that population and measure movement of individuals into the wooded area adjacent. A similar program was begun in the woods nearby with *L. appalachia*.

Samples of individuals of each species were captured live and stored for an electrophoretic analysis of genetic variation in the two groups, and for a measurement of their genetic similarity.

METHODS

Mark–recapture analysis of population size

A mark, release and recapture study was undertaken in what will henceforth be referred to as the Pothole area to estimate the density and turnover rate of the *L. eurydice* population there. The population was sampled 14 times during the period from 5 July to 22 July 1975. Insects were netted and secured in glassine envelopes during the 30 minute sampling period. Afterward, each insect captured for the first time was marked with a felt-tipped marker in a coded numerical fashion. The specimen number, sex, and condition of all newly-captured and recaptured insects were recorded; and the individuals were immediately released. The point of release was changed from one day to the next to avoid any overall displacement of individuals by capture. Most samples were taken in the early afternoon.

The data were analyzed using the method outlined by Jolly (1965). This method of analysis is particularly appropriate for use in studies involving three or more successive samples in a population where both dilution and loss are occurring (Southwood, 1966). The basic equation is:

$$\hat{P}_i = (\hat{M}_i n_i)/(r_i)$$

where $\hat{P}_i$ = the estimate of population size on day i, $\hat{M}_i$ = the estimate of the total number of marked animals in the population on day i, n_i = the number of individuals caught on day i, and r_i = the number of previously-marked animals caught on day i.

Other parameters of interest estimated by the Jolly method include $\hat{\phi}_i$ the probability of survival from release time on day i to capture on day $i + 1$, and $\hat{B}_i$, the number of new animals joining the population in the interval from i to $i + 1$ who are still alive at time $i + 1$.

Electrophoretic analysis of protein polymorphisms

Individuals of *Lethe eurydice* and *L. appalachia* were captured in the field during the period 5 July to 22 July 1975. All 173 individuals of *L.*

eurydice were collected in the Pothole area. Seventy-three individuals of *L. appalachia* were collected in several areas of concentration in the wooded areas of McLean Bogs Reserve. The boundaries of these concentrated areas were very diffuse, however, and individual *L. appalachia* specimens were often netted in other places.

The insects were frozen live after capture and stored at –80°C until electrophoresis. Specimens were prepared for electrophoresis by grinding, after removal of legs and wings, in 0.3 ml of a pH 7.0 buffer of 0.1 M tris, 0.001 M EDTA and 5×10^{-5} M NADP. The homogenates were drawn into capillary tubes, centrifuged at 10,000 rpm for 2 minutes and stored at –80°C.

Horizontal starch gel electrophoresis was performed on the soluble protein extracts by methods similar to those of Selander *et al.* (1971). The buffer systems used and the enzyme assays employed were as follows: lithium hydroxide (Selander *et al.*, 1971, buffer 2), glutamate-oxaloacetate transaminase (GOT), phosphohexose isomerase (PHI) and phosphoglucomutase (PGM); continuous tris-citrate (Selander *et al.*, 1971, buffer 4), malate dehydrogenase (MDH, 2 loci), α-glycerophosphate dehydrogenase (α-GPD), and isocitrate dehydrogenase (IDH, 2 loci). A total of eight enzyme-synthesizing loci were resolved. Five of these were polymorphic in *L. eurydice*, four were polymorphic in *L. appalachia* (a polymorphic locus was defined as one in which the most common allele occurred at a frequency of >0.99). Allele frequencies were estimated directly from the phenotype frequencies for each locus. The formula for heterozygosity of an individual locus is $H = 1 - \sum p_i^2$ where p equals the frequency of an allele at the locus.

An estimate of the genetic distance separating the two populations was made using the method of Nei (1975) which provides an estimate of the mean number of codon differences per structural gene locus. This index may take values from 0, representing populations with no alleles in common, to 1, representing populations with identical frequencies of the same alleles.

RESULTS

Mark–recapture analysis of population size

The calculated estimates of the parameters $\hat{P}_i$, $\hat{\phi}$, and $\hat{B}_i$ for each day are given in Table 1, and the daily estimates of population size ($\hat{P}_i$) are shown in Figure 1. These data indicate two distinct peaks in population size: one on 10 July and a second, smaller peak on 14 July. Using a method of graphical estimation (Southwood, 1966) the area under the population estimate curve was calculated and this total, divided by the

TABLE 1. Results of analysis of mark–recapture data.

Day (i)	Proportion of recaptures ($\hat{\alpha}_i$)	No. marked animals at risk ($\hat{M}_i$)	Survival rate ($\hat{\phi}_i$)	No. of new animals ($\hat{B}_i$)	Total population ($\hat{P}_i$)	Standard error of estimation $\left(\sqrt{V(\hat{P}_i/P)}\right)$
1	—	0	.553	—	—	0.0
2	.136	16.6	.736	98.3	122.1	72.5
3	.129	24.0	1.15	193.3	186.0	117.0
4	.152	57.5	.941	330.7	378.3	175.9
5	.111	75.8	1.68	166.1	682.9	396.4
6	.210	178.0	.296	–8.5	847.6	375.2
7	.250	60.3	.913	–15.9	241.2	139.0
8	.382	77.0	.410	223.6	201.6	70.5
9	.129	39.4	1.17	–30.9	305.4	202.3
10	.276	75.2	.136	28.8	272.5	111.1
11	.194	(13)	—	—	(65.7)	1.0
12	.093	(03)	—	—	(32.3)	2.0
13	.066	(01)	—	—	(15.2)	2.0
14	.000	(00)	—	—	—	—

average adult lifetime (derived from the mean survival rate $\hat{\phi}_i$), provided an estimate of total population size of 2,912 insects. No *L. eurydice* individuals were ever netted in the woods, nor were any *L. appalachia* caught in the Pothole area.

A similar mark–recapture program was begun with *L. appalachia* at a site in the woods about 100 meters from the *L. eurydice* population. This particular site was chosen because of the consistently higher densities of butterflies observed there in comparison to the woods in general. However, this study was abandoned because of an extremely low rate of recaptures and a general scarcity of *L. appalachia* individuals. In the first four days of the study, 29 specimens were netted, marked and released in 2 manhours of search; only one of these marked individuals was ever recaptured. It became apparent that the site represented only a temporary aggregation point for *L. appalachia,* and not a stable population unit. Individual insects tended to move extensively through large areas of the wooded bog basin, and several similar high-density sites were subsequently found.

Electrophoretic analysis of protein polymorphisms

The frequencies of all alleles for the eight enzyme-synthesizing gene loci for each species are shown in Table 2.

In *Lethe eurydice*, one locus (IDH-II) was represented by a single allele in all individuals. Two loci were dominated by single alleles with frequencies greater than 99 percent (GOT and α-GPD). Two loci were

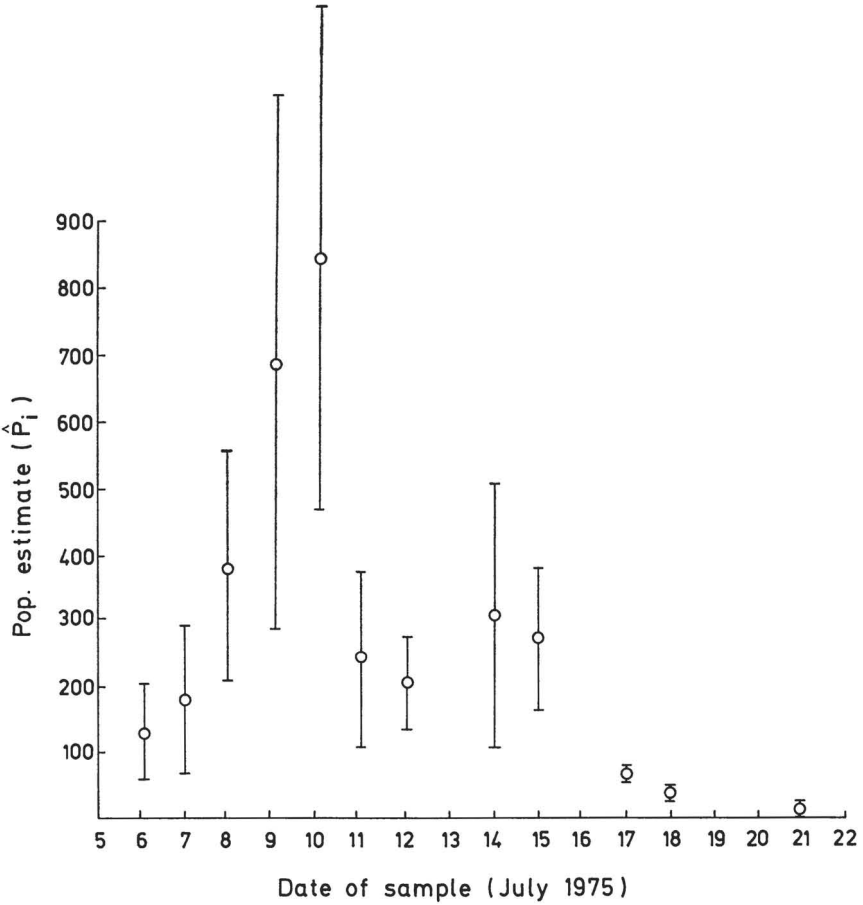

FIG. 1. Daily estimates of population size ($\hat{P}_i$) for *Lethe eurydice* at McLean Bogs Reserve, Pothole area.

dominated by single alleles with frequencies greater than 95 percent but less than 99 percent (MDH-I and MDH-II). Of the other three polymorphic loci, one locus had two alleles represented in the population (IDH-I), one locus had three alleles (PHI), and one locus had four alleles (PGM).

The mean heterozygosity for the population is 0.175. The heterozygosities of individual loci are listed in Table 3. The genotype frequencies at all loci were not signifcantly different from those predicted by the Hardy-Weinberg expression using the χ^2 goodness-of-fit test (Sokal and Rohlf, 1969).

Lethe appalachia showed a similar pattern of electromorphic variation.

TABLE 2. Results of analysis of allozyme variation.

Locus	Allele	*Lethe eurydice* Allele frequency	*Lethe eurydice* Sample size	*Lethe appalachia* Allele frequency	*Lethe appalachia* Sample size	Results of G-test ($p =$)
GOT	a'	—	320	.012	162	N.S.
	a	.977		.988		
	b	.003		—		
PHI	a	.103	340	—	164	<.001
	b	.679		.561		
	c	.240		.421		
	d	—		.018		
PGM	a	.321	308	.488	162	<.001
	b	.546		.265		
	c	.127		.204		
	d	.006		.043		
MDH-I	a	.034	320	.006	162	.036
	b	.966		.994		
MDH-II	a	.975	320	1.00	162	.01
	b	.025		—		
α-GPD	b	.993	320	1.00	162	N.S.
	c	.007		—		
IDH-II	a	.876	322	.988	162	<.001
	b	.122		.012		
IDH-II	a	1.00	226	1.00	150	N.S.

Three loci (MDH-II, α-GPD and IDH-II) were represented in the population by single alleles. One locus (MDH-I) was strongly dominated by an allele with a frequency greater than 99 percent. Two loci (GOT and IDH-I) were dominated by single alleles with frequencies between 95 and 99 percent. The remaining two loci had three (PHI) and four (PGM) alleles present in the population.

TABLE 3. Estimates of heterozygosity per locus.

Locus	*Lethe eurydice*	*Lethe appalachia*
GOT	.006	.024
PHI	.470	.508
PGM	.583	.648
MDH-I	.066	.012
MDH-II	.048	.000
α-GPD	.014	.000
IDH-I	.212	.024
IDH-II	.000	.000
Mean	.175	.152

The mean heterozygosity of the *L. appalachia* population was 0.152. Heterozygosities of individual loci can be found in Table 3. No locus showed significant deviation from Hardy-Weinberg expectation using the χ^2 goodness-of-fit test.

The two species are highly significantly different ($p < .01$, G-test) in allele frequencies at four of the eight loci examined, and significantly different ($p = .036$) at another. These data clearly indicate reproductive isolation in sympatry. Using the genetic distance measure of Nei (1975) the distance separating these species equals 0.145, a figure well within the rather wide range of available estimates of distances separating sibling pairs. (Nei's measure can range between a maximum distance of 1.00, representing no alleles in common, to a minimum of 0.0, representing total identity.)

A very small number of individuals (six) of *Lethe portlandia* captured at the McLean Bogs Reserve were analysed at the same eight enzyme loci. Although the sample size was insignificant for statistical purposes, the electromorphs at six of the loci tested represented clearly different mobility classes from those present in either *L. appalachia* or *L. eurydice*.

DISCUSSION

The results of this study fully agree with the conclusion of Cardé, Shapiro and Clench (1970) and Shapiro and Cardé (1970) that the butterflies *Lethe eurydice* and *L. appalachia* are distinct, although very similar, sibling species. Both the mark-recapture study and the analysis of genetic variation demonstrate that the two are genetically isolated and habitat-segregated, at least in the locality studied.

The mark–recapture study indicates that the two species strongly resist crossing over from the open to the wooded habitat, or vice versa. It also suggests that the two populations have contrasting spatial structure. *Lethe eurydice* occupies a small, isolated, concentrated, uniform patch of acceptable habitat, while *L. appalachia* occupies a more "fine-grained" habitat, moving extensively between more fragmented and diffuse sites of maximum acceptability.

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NOTES AND NEWS

THE JAMES H. BAKER COLLECTION

The James H. Baker collection of insects has been received at the Department of Entomology, Smithsonian Institution. Baker's material consists of slightly more than 24,400 specimens, primarily Lepidoptera, but also contains many Coleoptera and Diptera.

Among the Lepidoptera the collection is especially rich in Geometridae, Baker's specialty. Most of the specimens in this collection are from eastern Oregon, but Baker enjoyed a wide correspondence and traded considerably; consequently there is a rather liberal sprinkling of moths and butterflies from localities other than Baker's home state. Baker also collected in such places as Arizona, Idaho, and Nevada, so there is a nice representation of species from those areas.

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