GENIC HETEROZYGOSITY AND VARIATION IN PERMANENT TRANSLOCATION HETEROZYGOTES OF THE *OENOTHERA BIENNIS* COMPLEX

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ABSTRACT

Genic heterozygosity and variation were studied in the permanent translocation heterozygotes *Oenothera biennis* I, *Oe. biennis* II, *Oe. biennis* III, *Oe. strigosa, Oe. parviflora* I, *Oe. parviflora* II, and in the related bivalent formers *Oe. argillicola* and *Oe. hookeri*. From variation at 20 enzyme loci, we find that translocation heterozygosity for the entire chromosome complex is accompanied by only moderate levels of genic heterozygosity: 2.8% in *Oe. strigosa*, 9.5% in *Oe. biennis* and 14.9% in *Oe. parviflora*. Inbred garden strains of *Oe. argillicola* exhibited 8% heterozygosity; neither garden nor wild strains of *Oe. hookeri* displayed heterozygosity and only a single allozyme genotype was found. The mean number of alleles per locus is only 1.30 in *Oe. strigosa*, 1.40 in *Oe.* biennis, and 1.55 in *Oe. parviflora*, compared to 1.40 in *Oe. argillicola*. Clearly, the ability to accumulate and/or retain heterozygosity and variability has not been accompanied by extraordinary levels of either. Clinal variation is evident at some loci in each ring-former. A given translocation complex may vary geographically in its allozymic constitution. From gene frequencies, *Oe. biennis* I, II, and III, *Oe. strigosa* and *Oe. hookeri* are judged to be very closely related, whereas *Oe. argillicola* seems quite remote; *Oe. parviflora* is intermediate to the two phylads. Gene frequencies also suggest that *Oe. argillicola* diverged from the Euoenothera progenitor about 1,000,000 years ago, whereas most of the remaining evolution in the complex has occurred within the last 150,000 years.

PERMANENT translocation heterozygosity is a genetic *tour de force* which has evolved in subgenera of Oenothera (reviewed by CLELAND 1972), species of Gaura (BHADURI 1942; RAVEN and GREGORY 1972), Gayophytum (LEWIS and SZWEYKOWSKI 1964), *Hypericum punctatum* (HOAR 1931), and *Rhoeo discolor* (SAX 1931, 1935; WALTERS and GERSTEL 1948; LIN 1973), and in populations of *Paeonia californica* and *P. brownii* (WALTERS 1942; STEBBINS and ELLERTON 1939) and *Isotoma petraea* (JAMES 1965, 1970). There is general agreement that complex structural hybridity arose as a mechanism that preserves or accumulates genic heterozygosity in unispecific or hybridizing populations originally adapted to outbreeding but more recently subjected to inbreeding by virtue of self-compatibility or small population size (CLELAND 1936; DARLINGTON

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1937, 1958; CLELAND, PREER and GECKLER 1950; STEBBINS 1950, 1971). Therefore, relative to bivalent formers, structurally heterozygous species ought to be highly heterozygous genically and, correlatively, should exhibit a large proportion of polymorphic loci with a rich array of allelic variants.

In contrast to an abundance of theory, there is no information on the levels of genic variation and heterozygosity in population systems with permanent translocation heterozygosity. Do such species contain an unusual store of genetic variability and high levels of heterozygosity relative to their bivalent-forming congeners, or plant species in general? To what extent do related structurally heterozygous species differ in genic polymorphism and heterozygosity, and to what extent do these parameters vary within species? Are alternate alleles distributed randomly in space or are clines present? What is the degree of genic variation within a given chromosome complex?

We have turned to the *Oenothera biennis* complex within the subgenus Euoenothera for answers to these questions. The complex is composed of three bivalent-forming cross-pollinating species (*Oe. hookeri*, *Oe. grandiflora* and *Oe. argillicola*) and three self-pollinating species (*Oe. biennis*, *Oe. strigosa* and *Oe. parviflora*) (cf. CLELAND 1972). In the self-pollinating species, all chromosomes are heterozygous for translocations, resulting in a ring of 14 chromosomes at diakinesis. Chiasmata are terminal and chromosome disjunction is alternate so that all chromosomes and essentially all genes of each 7-chromosome "Renner" complex come to reside in the same gamete. A system of balanced lethals and incompatibility alleles prevents the formation of structural homozygotes. As a consequence of these features, the structural heterozygotes in these selfing Oenothera have the maximum amount of linkage attainable in a sexually reproducing organism.

CLELAND (1972) contends that the complex is derived from five outbreeding ancestral stocks, referred to as Populations, which developed in Mexico and Central America and spread in successive waves across North America. The phylogeny is depicted in Figure 1. CLELAND's ancestral Population 1 was the first to invade the United States, and is represented today by the Appalachian shale barren endemic *Oe. argillicola*. Population 1 and *Oe. argillicola* bear some resemblance to members of the subgenus Raimannia. Population 2 then spread northward and hybridized with Population 1 yielding *Oe. parviflora* I. A relict of Population 2 grows near Mobile, Alabama and is known as *Oe. grandiflora*. Subsequently Populations 3 and 4, which were more xerophytic than Populations 1 and 2, migrated northward into the central and eastern United States. Population 3 hybridized with Population 1 producing *Oe. parviflora* II; both races of *Oe. parviflora* occur in the northeastern United States and across southern Canada to the Great Lakes. Population 3 also hybridized with Population 2 to produce *Oe. biennis*. This species diverged into race I which grows throughout much of the eastern United States excluding New England, and race II whose range is like that of *Oe. parviflora*. *Oenothera biennis* I and II hybridized to produce race III, which is restricted largely to the central Appalachian region. Population 3 also hybridized with Population 4 at a later time to produce *Oe.*

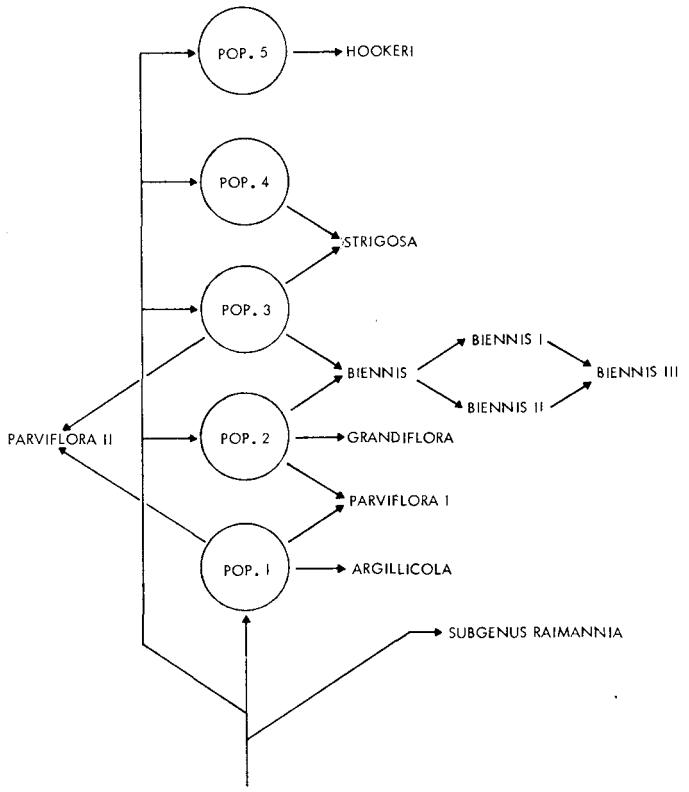

FIGURE 1.—Putative phylogeny in the *Oe. biennis* complex (original and after CLELAND 1972).

strigosa, which extends from the Mississippi River to the Pacific Coast excluding the southwestern portion of the country. This area is the province of *Oe. hookeri* which is a derivative of Population 5. Although not involved in the parentage of the ring-formers, this species hybridizes with *Oe. strigosa*.

In addition to addressing the general questions posed above, we consider questions which relate specifically to Oenothera phylogeny. Are the alleles present in the structural heterozygotes also present in the structural homozygotes with which they share common ancestry? Is there a relationship between the age of the ancestral populations and the level of genic polymorphism and heterozygosity in the structural heterozygotes? Does the genetic similarity of strains within taxa decrease as the geographic distance between the collection sites increases?

MATERIALS AND METHODS

A total of 184 cultivated strains derived from wild populations throughout the ranges of eight Oenothera taxa was studied. These had been maintained via self-fertilization by PROFESSOR RALPH CLELAND for periods up to four decades and served as the source of his extensive cytogenetic analyses. The taxa (and the respective number of strains) studied were as follows: *Oe. biennis* I (57), *Oe. biennis* II (43), *Oe. biennis* III (6), *Oe. strigosa* (29), *Oe. parviflora I* (19),

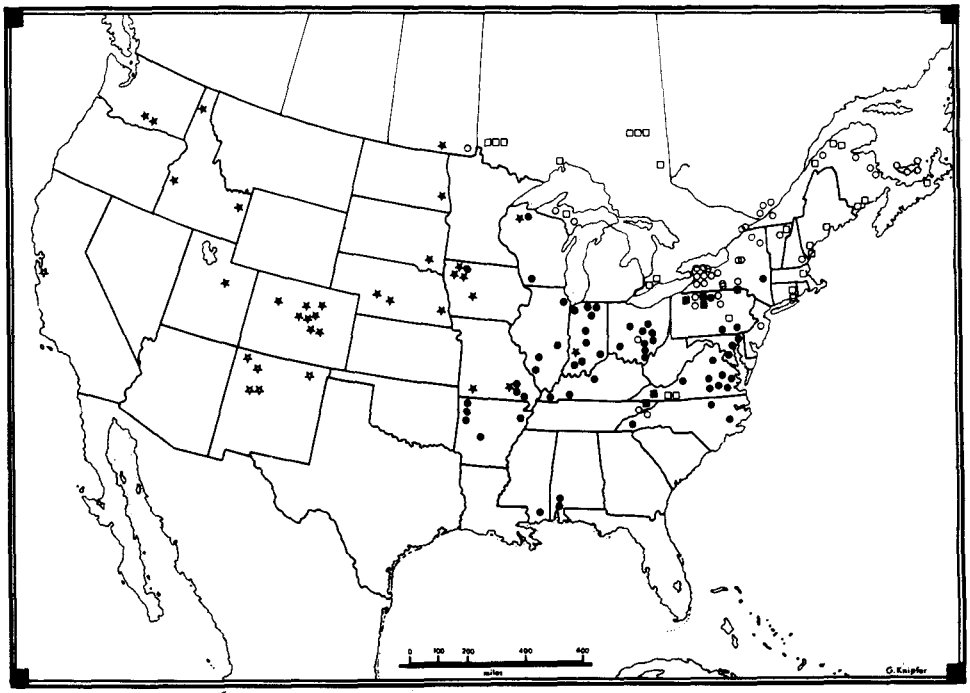

FIGURE 2.—Collection sites for cultivated strains of *Oe. hookeri* (open stars), *Oe. strigosa* (closed stars), *Oe. biennis* I (closed circles), *Oe. biennis* II (open circles), *Oe. biennis* III (closed squares), and *Oe. parviflora* (open squares).

Oe. parviflora II (10), *Oe. argillicola* (10), *Oe. hookeri* (10). The locations of the collection sites are shown in Figure 2.

Four wild populations of *Oe. hookeri* from California were also studied. The collection sites were: south of Lompoc, Santa Barbara Co.; Santa Ynez Mountain, Santa Barbara Co.; Santa Cruz Island; and San Francisco, San Francisco Co. Twenty-eight plants from each population were analyzed.

It must be noted that the cultivated strains represent more than a mere sampling of variability in geographically widespread individuals. Extensive intrapopulation analysis of *Oe. biennis* (LEVIN, HOWLAND and STEINER 1972; LEVIN 1975b) demonstrates that populations are genetically depauperate, exhibiting only one or a few allozyme phenotypes. For the ring-formers, consequently, allozyme variation in the cultivated strains represents nearly all variability manifest in the respective source populations.

Genic variation and heterozygosity were determined via allozyme analysis. Two-week-old seedlings were homogenized in 50 μl of extraction buffer (0.5 ml mercaptoethanol in 100 ml 0.5 M phosphate, pH 7.0) and applied in a single sample slot to a horizontal-type starch gel. Electrophoresis was conducted horizontally in 12.5% starch gels prepared with either a LiOH discontinuous buffer (SELANDER *et al.* 1971) or a Tris-EDTA-borate continuous buffer (RUDDLE and NICHOLS 1971). A dilute solution of bromphenol-blue was added to extracts partitioned on the latter buffer system to mark the electrophoretic front. Control strains were run on all gels as internal standards.

A total of 11 enzyme species was assayed. Glutamate oxalate transaminase (GOT), leucyl-tyrosine peptidase (LTP), leucyl-glycyl-glycine peptidase (LGGP), valyl-leucine peptidase (VLP), and malate dehydrogenase (MDH) were assayed on LiOH gels. Glucose-6-phosphate dehydrogenase (G-6PD), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), phospho-

glucose isomerase (PGI), acid phosphatase (ACP), and alkaline phosphatase (ALP) were assayed on Tris-EDTA-borate gels. Staining procedures for GOT were from DeLorenzo and Ruddle (1970), for LTP, LGGP, and VLP from Ruddle and Nichols (1971), for MDH and PGI from Selander *et al.* (1971), and for G-6PD, PGM, ACP, and ALP from Shaw and Prasad (1970). The LAP assay required incubation for 1 hr in a solution of 10 mg L-leucyl-beta-naphthylamide HCl, 2.5 ml dimethyl formamide, and 47.5 ml 0.1 M phosphate buffer, pH 7.0, rinsing after incubation, and staining with 50 mg Black K salt in 50 ml of the phosphate buffer. All systems migrated anodally.

In the sections that follow, the abbreviations used to designate each enzyme species are written in capital letters. When more than one form of the enzyme exists, each controlled by a separate locus, a hyphenated numeral is added to the italicized symbol of the locus. The locus encoding the form with the least anodal migration is called one, the next two, etc. Alleles are denoted by alphabetical superscripts.

Allozyme genetics were judged from segregation patterns manifest in F_1 reciprocal hybrids of *Oe. hookeri* or *Oe. grandiflora* and one of 22 ring-forming strains (Levy, Steiner and Levin 1975). Alleles are inherited as co-dominant markers, and are transmitted either through the egg or sperm in a given cross. We judge the enzymes studied to be controlled by 20 loci.

RESULTS

Genetic polymorphism: Allozyme variation in the *Oe. biennis* complex is rather limited in view of the genetic system and number of taxa studied. Of the 20 loci considered, the following nine are monomorphic and share the same allele in all taxa: *G-6pd, Lggp-1, Lggp-2, Ltp-1, Acp-1, Acp-2, Alp-1, Alp-3* and *Mdh.* Three loci, *Pgm-2, Got-2,* and *Alp-2,* are monomorphic within taxa but differ between taxa. The components of the *Oe. biennis* complex are polymorphic at only the following eight loci: *Pgm-1, Vlp, Got-1, Ltp-2, Ltp-3, Pgi-1, Pgi-2,* and *Lap* (Table 1). In comparisons among the taxa, first we should note that the Cleland strains of *Oe. hookeri* and the four population samples we have studied have only one genotype. This Oenothera is the only one lacking polymorphic loci. The proportion of polymorphic loci varies from .20 in *Oe. argillicola* to .25 in *Oe. strigosa,* .30 in each of the races of *Oe. biennis,* and .40 in each race of *Oe. parviflora.* The relatively small amount of variability in the species complex is most evident when one considers that the proportion of polymorphic loci within a *single population* of each of the self-fertilizing grasses *Avena fatua* and *A. barbata* is .54 and .31, respectively (Marshall and Allard 1970) and that within the outbreeding *Liatris cylindracea* is .56 (Schaal 1974).

Allelic diversity within the species complex is also meager. The mean number of alleles per polymorphic locus ranges from 2.20 in *Oe. strigosa* to 2.25 in *Oe. argillicola* and *Oe. parviflora* II, and 2.33 in each of the races of *Oe. biennis* and *Oe. parviflora* I; the grand mean is 2.30, indicating that generally there are only two alternative alleles at any polymorphic locus throughout the entire range of each taxon. In terms of total allelic diversity the mean number of alleles per locus ranges from the lower limit of 1.00 in *Oe. hookeri* to a maximum value of 1.55 in *Oe. parviflora* I; the grand mean is 1.36 (Table 2).

Gene frequencies at the eight polymorphic loci of the complex are summarized in Table 1. Although *Oe. biennis* I and II have the same genomic constitution (biennis and strigosa genomes) the races differ in gene frequency at all of their

TABLE 1

*Summary of gene frequencies at polymorphic loci in Oenothera**

Locus	Allele	Taxon† bI	bII	bIII	st	pI	pII	hk	arg
Pgm-1	a	.11	.04	.08	.10				
	b	.84	.94	.84	.90			1.0	
	c	.05	.02	.08		.03	.05		
	d					.09	.20		
	e					.88	.75		.15
	f								.85
Pgm-2	a	1.0	1.0	1.0	1.0			1.0	
	b					1.0	1.0		
	c								1.0
Vlp	a	.35	.13	.25	.03				
	b	.49	.62	.33	.25	.16	.20		.05
	c	.16	.25	.42	.72			1.0	
	d					.11			.15
	e					.73	.80		.80
Got-1	a	.55	.74	.75	.95			1.0	
	b	.45	.26	.25	.05				
	c					.47	.61		.35
	d					.53	.39		.65
Got-2	a	1.0	1.0	1.0	1.0			1.0	
	b					1.0	1.0		1.0
Ltp-2	a	.10	.19	.08					
	b	.90	.81	.92	1.0			1.0	
	c					.21	.25		.35
	d					.79	.75		.65
Ltp-3	a	1.0	1.0	1.0				1.0	
	b					.16	.15		1.0
	c					.84	.85		
Pgi-1	a	.07	.14	.25	.02				
	b	.93	.86	.75	.98			1.0	
	c								1.0
	d					.18	.15		
	e					.82	.85		
Pgi-2	a	.79	.79	.83	.89	.89	.85	1.0	
	b	.21	.21	.17	.11	.11	.15		
	c								1.0
Lap	a	1.0	1.0	1.0	1.0			1.0	
	b					.11	.05		
	c					.58	.80		1.0
	d					.32	.15		
Alp-2	a	1.0	1.0	1.0	1.0			1.0	
	b					1.0	1.0		1.0

* The following loci were monomorphic: *G-6pd, Lggp-1, Lggp-2, Ltp-1, Acp-1, Acp-2, Alp-1, Alp-3, Mdh.*

† Abbreviations: b = biennis, st = strigosa, p = parviflora, hk = hookeri, arg = argillicola.

TABLE 2

Summary of genetic properties of Oenothera taxa

Taxon	Proportion polymorphic loci	Mean number alleles per locus	Mean % heterozygosity	No. strains
Oe. biennis I	.30	1.40	9.1	57
Oe. biennis II	.30	1.40	10.0	43
Oe. biennis III	.30	1.40	10.8	6
Oe. strigosa	.25	1.30	2.8	29
Oe. parviflora I	.40	1.55	14.7	19
Oe. parviflora II	.40	1.50	15.0	10
Oe. hookeri	0	1.00	0	14*
Oe. argillicola	.20	1.40	8.0	10

* Denotes number of collection sites.

polymorphic loci. An analysis of variance following arcsin transformation demonstrates that the races differ significantly in the frequencies of *Pgm-1*^{a} ($F_s = 4.06$, $P < .05$), *Vlp*^{c} ($F_s = 18.29$, $P < .01$), *Got-1*^{a} ($F_s = 7.41$, $P < .01$), *Ltp-2*^{a} ($F_s = 5.13$, $P < .01$), and *Pgi-1*^{a} ($F_s = 3.76$, $P < .05$). No significant differences in gene frequency were found between *Oe. biennis* III and either of its conspecific races. *Oenothera strigosa* and *Oe. biennis* (I and II) are divergent in a number of respects although they share a common genome. Firstly, *Oe. strigosa* is less variable, having fewer alleles at *Pgm-1* and *Ltp-2*; all other alleles are in common. Secondly, the two species differ significantly in the frequencies of *Vlp*^{c} ($F_s = 40.17$, $P < .001$), *Got-1*^{b} ($F_s = 18.11$, $P < .001$), and *Pgi-1*^{a} ($F_s = 5.09$, $P < .01$). The remaining structural heterozygote, *Oe. parviflora* (I and II) is quite different from the others in its genetic makeup. With regard to the polymorphic loci, *Oe. parviflora* shares both alleles at *Pgi-2* and one allele at *Pgm-1* and *Vlp* with the other ring-formers but has two distinctive alleles at each of the latter two loci, and is completely distinctive at *Got-1*, *Ltp-2*, *Ltp-3*, *Pgi-1*, and *Lap*. At monomorphic loci the ring-formers share common alleles except at *Pgm-2*, *Got-2*, and *Alp-2*, where *Oe. parviflora* exhibits distinctive alleles. The only significant difference between the races of *Oe. parviflora* is for *Lap*^{b} ($F_s = 9.83$, $P < .01$).

The bivalent formers are quite dissimilar in their allozyme constitution (Table 1). *Oenothera hookeri* and *Oe. argillicola* share no alleles at the eight polymorphic loci. In comparing *Oe. hookeri* with *Oe. biennis* and *Oe. strigosa*, it is evident that the allele for which *Oe. hookeri* is fixed is the predominant allele in *Oe. biennis* and *Oe. strigosa*.

Regressions of gene frequencies on north-south and east-west geographical axes were performed for each polymorphic locus in *Oe. biennis* I and II, *Oe. strigosa* and *Oe. parviflora*. Geographical coordinates were determined in a strict linear fashion, rather than by latitude and longitude measures, with a scale of 5.5 miles separation between adjacent coordinates. Seven instances of clinal variation were revealed in the total of 48 regressions. The significant results are summarized in Table 3. In *Oe. biennis* I, *Pgm-1*^{b} increases in mean frequency from .70 in the

TABLE 3

Summary of significant east-west and north-south regressions for gene frequencies in Oenothera

Taxon	Direction	Gene allele	Correlation coefficient	F_s
Oe. biennis I	W to E	*Pgm-1*^{b}	+.312	5.516*
Oe. biennis I	W to E	*Vlp*^{c}	+.385	8.859**
Oe. biennis II	S to N	*Got-1*^{a}	+.311	3.862*
		Got-1^b	—.311	3.862*
Oe. strigosa	S to N	*Pgm-1*^{a}	+.392	4.547*
		Pgm-1^b	—.302	4.547*
Oe. strigosa	W to E	*Got-1*^{a}	+.429	5.661*
		Got-1^b	—.429	5.661*
Oe. parviflora	S to N	*Got-1*^{d}	+.552	9.223**
		Got-1^c	—.552	9.223**
Oe. parviflora	S to N	*Pgi-1*^{e}	+.460	5.693*
		Pgi-1^d	—.460	5.693*

* Denotes significance at 5% level.
** Denotes significance at 1% level.

most western strains to .95 in the east (see Figure 2) and *Vlp*^{c} increases in mean frequency from .05 to .35 in a parallel fashion. A cline corresponding to latitude is manifest in *Oe. biennis* II; *Got-1*^{a} increases in mean frequency from .70 in the most southern strains to .95 in the north with a corresponding clinal reduction of *Got-1*^{b}. One latitudinal and one longitudinal cline are manifest in *Oe. strigosa*. *Got-1*^{a} increases in mean frequency from .83 in the west to 1.0 in the east and *Pgm-1*^{a} increases in mean frequency from .07 in the south to .19 in the north. The alternative alleles at each locus, *Got-1*^{b} and *Pgm-1*^{b}, decrease correspondingly. Two north-south clines are manifest in *Oe. parviflora*; both races are treated jointly because they are sympatric and share similar gene frequencies. From south to north *Got-1*^{d} increases in mean frequency from .17 to .55 and *Pgi-1*^{e} from .67 to .95. The alternative alleles at each locus, *Got-1*^{c} and *Pgi-1*^{d}, decrease correspondingly.

Heterozygosity: All strains in this investigation were maintained by CLELAND through periodic self-fertilization, and many have undergone at least 20 generations of selfing (STEINER, personal communication). The permanent translocation heterozygotes experience no loss of heterozygosity in the process, whereas the bivalent formers would be expected to lose about 50% of their heterozygosity each generation. Consequently the inbred, bivalent-forming strains may not reflect levels of heterozygosity in natural populations. Such qualification apparently is unnecessary for *Oe. hookeri* values since only a common, homozygous allozyme genotype is manifest in both wild populations and inbred samples. However, the mean heterozygosity (for all loci averaged over all strains) of *Oe. argillicola* is 8.0%. Ostensibly natural selection or conscious selection by CLELAND and associates for vigorous representatives of a strain is responsible for the retention of heterozygosity. Heterozygosity in *Oe. argillicola* may well be greater

in natural populations than what we have observed, and this should be kept in mind when the significance of genic heterozygosities of the translocation heterozygotes is considered.

The mean heterozygosity for all taxa considered is presented in Table 2. For the ring-formers, the mean heterozygosity increases with the proportion of polymorphic loci, but the differences in heterozygosity which accompany small shifts in polymorphic loci are quite striking. With 25% polymorphic loci, the mean heterozygosity of *Oe. strigosa* is only 2.8%. Most populations are homozygous for all loci considered, the maximum level of heterozygosity being only 10% (Figure 3). With 30% polymorphic loci, the mean heterozygosity of *Oe. biennis* (all races) is 9.5%. Heterozygosity per strain varies from 0% to 20% in race I and 0% to 25% in race II (Figure 3). Of the six strains of race III examined three displayed 20% heterozygosity, two total homozygosity, and one 5% heterozygosity. *Oenothera parviflora* (both races) has 40% polymorphic loci and a mean heterozygosity of 14.9%. Heterozygosity per strain varies from 5% to 35% (Figure 3). The significance of the differences in heterozygosity between the structural heterozygotes was tested employing the Mann-Whitney U test (SOKAL and ROHLF 1969, p. 393). The differences between races within *Oe. biennis* and *Oe. parviflora* are not significant; otherwise all differences are statistically significant. Note that mean heterozygosity in *Oe. strigosa* has not surpassed that of the inbred stocks of *Oe. argillicola*.

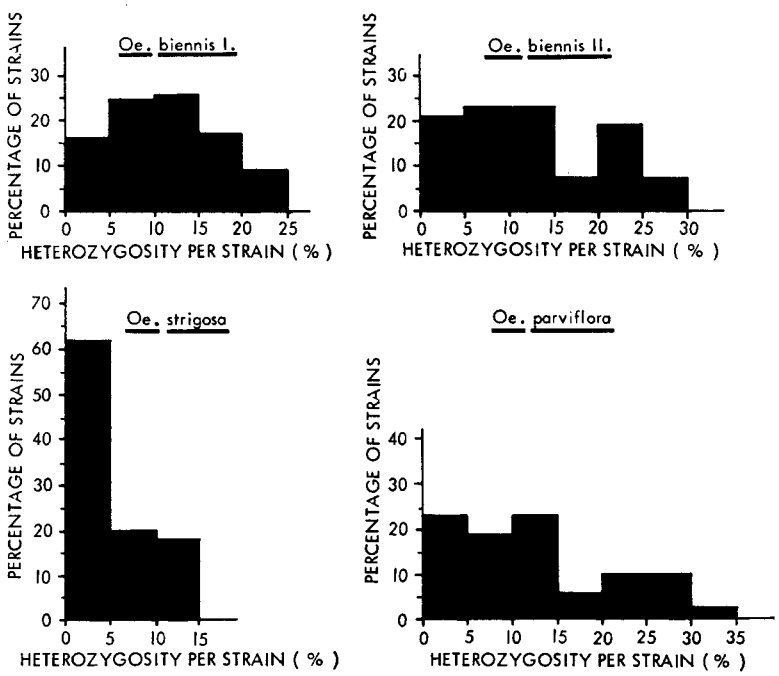

FIGURE 3.—The distribution of heterozygosity percentages in strains of *Oe. biennis* I and II, *Oe. strigosa* and *Oe. parviflora*.

The possibility of clinal variation of heterozygosity within taxa was explored by regressing heterozygosity against geographical coordinates as before. No significant correlations were observed in *Oe. biennis* I or II and *Oe. parviflora* (both races collectively), nor did any approach the 10% level.

Because egg and sperm chromosome complexes undergo negligible genic recombination, there may be pronounced allele frequency differences between them. We may gain insight into this matter by comparing the observed heterozygosity at a locus with that expected from allele frequencies, assuming no selection, random mating, and random distribution of alleles (within each complex) throughout the range of a taxon. An excess of heterozygotes would suggest that the egg and sperm complexes differ in allele frequency. A deficiency of heterozygotes would suggest that the complexes had similar allele frequencies and that within each complex alternate alleles were not distributed randomly in space. Good correspondence between observed and expected number of heterozygotes would not be definitive since such a result might accrue either from similar allele frequencies or from different frequencies whose effect is counterbalanced by a nonrandom distribution.

The observed and expected number of heterozygotes at polymorphic loci in *Oe. biennis*, *Oe. strigosa*, and *Oe. parviflora* are presented in Table 4. A chi-square goodness-of-fit test was performed on the genotype arrays at each locus to determine whether the heterozygote departure was statistically significant. The corre-

TABLE 4

Observed and expected number of heterozygotes in ring-forming oenotheras

Locus		Taxon bI	bII	st	pI	pII
Pgm-1	Obs.*	15.0	5.0	6.0	4.0	4.0
	Exp.†	17.1	5.2	5.2	4.1	4.0
Vlp	Obs.	35.0	14.0‡	2.0‡	4.0	0.0
	Exp.	35.3	22.8	12.1	8.2	3.2
Got-1	Obs.	16.0‡	19.0	3.0	11.0	8.0§
	Exp.	28.2	16.5	2.8	9.5	4.8
Ltp-2	Obs.	11.0	17.0	—	8.0	5.0
	Exp.	10.3	13.2	—	6.3	3.8
Ltp-3	Obs.	—	—	—	6.0	3.0
	Exp.	—	—	—	5.1	2.6
Pgi-1	Obs.	8.0	13.0	1.0	3.0	3.0
	Exp.	7.4	10.4	1.1	5.6	2.6
Pgi-2	Obs.	19.0	18.0	4.0	4.0	3.0
	Exp.	18.9	14.3	5.7	3.7	2.6
Lap	Obs.	—	—	—	16.0§	4.0
	Exp.	—	—	—	10.8	3.4

* Denotes observed number of heterozygotes.

† Denotes number of heterozygotes expected from gene frequencies assuming random association of alleles.

‡ Denotes significant deficiency of heterozygotes, $P \leq .05$.

§ Denotes significant excess of heterozygotes, $P \leq .05$.

spondence between expected and observed values is good for most loci in each of the taxa. Significant heterozygote excesses occur only at *Lap* (chi-square = 9.88, $P < .01$) in *Oe. parviflora* I and at *Got-1* (chi-square = 6.00, $P < .05$) in *Oe. parviflora* II. It is not surprising that heterozygote excess is manifest in *Oe. parviflora* because its egg and sperm complexes have quite different morphological syndromes (CLELAND, PREER and GECKLER 1950; CLELAND 1972). Heterozygote deficiencies appear at one locus in each taxon of the other ring-formers. There is a deficiency at *Got-1* (chi-square = 8.68, $P < .05$) in *Oe. biennis* I, at *Vlp* (chi-square = 14.39, $P < .05$) in *Oe. biennis* II, and at *Vlp* (chi-square = 37.60, $P < .01$) in *Oe. strigosa*. In each case we might surmise that allele frequencies of the egg and sperm complexes are similar and that alternate alleles are nonrandomly distributed in space, the pattern of distribution being parallel in both complexes. However, in view of the number of loci analyzed it is possible that one significant excess or deficiency per taxon may arise simply by chance. Consequently, the heterozygote departure tests are inconclusive in resolving major allele frequency differences between paired egg and sperm complexes. Nonetheless, the tests do indicate that structural heterozygosity does not necessarily maintain genic heterozygosity in excess of that produced in outbreeding bivalent-forming species.

Genetic affinity: To obtain a quantitative measure of the genetic differences between strains of the same taxa, and between different taxa, we have computed the normalized genetic identity proposed by NEI (1972). Populations within each of the ring-forming taxa are weakly differentiated. The mean genetic identity ($\bar{I}$) between all pairs of populations of *Oe.* strigosa is .97, as compared to .95 in *Oe. biennis* I and *Oe. biennis* II. The greatest within-taxon differentiation is found among populations of *Oe. parviflora*, which have a mean genetic identity of .92.

Having the genetic identity of all pairs of strains as well as the site of origin of each strain, we tested for correlations between the geographical distance between strains and their genetic identity. Despite the occurrence of clinical variation at some loci, none of the taxa displayed declining genetic identity with increasing distance. The closest case is in *Oe. biennis* II where the correlation coefficient $r = -.07$ ($F_s = 3.36$, $P < .08$).

The genetic identities of the taxa in all pairwise combinations are presented in Table 5. The greatest identities are between races of *Oe. biennis*; in each combination the identity exceeded .99. *Oe. strigosa* is very similar to *Oe. biennis*, having genetic identities in excess of .97 with all races. It is interesting to note that the greatest similarity was in combination with biennis III which, unlike races I and II, carries no dose of the strigosa genome and is separated from the present range of *Oe. strigosa* by over 1000 miles. *Oenothera hookeri* is closely allied to *Oe. biennis* and *Oe. strigosa*, as shown by genetic identities in excess of .85 in all combinations. *Oenothera parviflora* I and II are very similar ($\bar{I} = .99$) even though they are considered to have only one ancestor in common. *Oenothera argillicola* and *Oe. parviflora* are rather similar in genetic constitution ($\bar{I} = .81$) but clearly removed from the other taxa. Neither species in combination with *Oe.*

TABLE 5

Mean genetic identity (Ī) *between taxa of the* Oe. biennis *complex*

	bI	bII	bIII	Taxon st	hk	pI	pII
Oe. biennis II	.994						
Oe. biennis III	.993	.994					
Oe. strigosa	.978	.985	.991				
Oe. hookeri	.958	.962	.976	.995			
Oe. parviflora I	.559	.558	.561	.547	.535		
Oe. parviflora II	.549	.548	.551	.537	.525	.994	
Oe. argillicola	.495	.493	.495	.479	.468	.809	.817

biennis, *Oe. strigosa* or *Oe. hookeri* has a genetic identity which exceeds .56. Of the two, *Oe. parviflora* is consistently more similar to these taxa than is *Oe. argillicola*. This is to be expected since both races of *Oe. parviflora* ostensibly have one progenitor in common with *Oe. strigosa* and/or *Oe. biennis* (Figure 1).

In view of the restrictions placed on the mobilization of genetic variation in the ring-forming oenotheras, it is of heuristic value to assess genetic affinities based upon genotypes rather than gene frequencies. For this purpose we have employed HEDRICK's (1971) probability of genotype identity. The values (based on the 11 polymorphic loci in the group) for all combinations of taxa are presented in Table 6. We see a strong affinity between *Oe. biennis* I and II and *Oe. strigosa* with probabilities in excess of .90. However, *Oe. hookeri* now is somewhat removed from this aggregate by virtue of its lacking some of the alleles shared by the other taxa. The two races of *Oe. parviflora* are very similar and collectively with *Oe. argillicola* form a small ensemble of related taxa highly divergent from the remainder of the complex. The probability of identity between members of the *Oe. biennis* cluster and that containing genotypic *Oe. parviflora* and *Oe. argillicola* averages less than .10. In fact, *Oe. argillicola* has no genotypes in common with *Oe. biennis*, *Oe. strigosa*, or *Oe. hookeri*. The HEDRICK and NEI measures are basically in accord as far as species groups are concerned, but differ dramatically in the extent to which the species and groups have diverged.

TABLE 6

Probability of genotypic identity between taxa of the Oe. biennis *complex**

	bI	bII	Taxon st	hk	pI	pII
Oe. biennis II	.946					
Oe. strigosa	.901	.919				
Oe. hookeri	.697	.706	.801			
Oe. parviflora I	.095	.099	.089	0.000		
Oe. parviflora II	.090	.091	.092	0.000	.946	
Oe. argillicola	0.000	0.000	0.000	0.000	.545	.515

* Values not determined for *Oe. biennis* III due to small sample size.

DISCUSSION

Allozyme variation analysis does not provide strong evidence favoring heterozygote advantage as the prime stimulus for the evolution of complex structural hybridity in Oenothera. When compared to plant species in general, the permanent translocation heterozygotes of the *Oe. biennis* complex are genically depauperate and display only moderate levels of genic heterozygosity. Indeed, many strains of each structural heterozygote are completely homozygous in allozyme genotype (see Figure 3). The mean number of alleles per locus in the species studied here is 1.36, in contrast to 1.39 in the primarily asexual *Lycopodium lucidulum* (LEVIN and CREPET 1973) and 3.12, 1.84, 2.12, and 2.05 in the outbreeding *Lupinus texensis*, *L. subcarnosus*, *Hymenopappus scabiosaeus*, and *H. artemisiaefolius*, respectively (BABBEL and SELANDER 1974). It should also be noted that the latter values represent only a small sample of the range of each species and, therefore, are probably underestimates of the total allozyme diversity. Collectively, mean heterozygosities of the ring-formers are similar to those reported in other plants. Among self-fertilizing species, mean heterozygosity ranges from 1% in *Phlox cuspidata* (LEVIN 1975a) to ca. 3% in *Avena barbata* (MARSHALL and ALLARD 1970). Among self-incompatible species, mean heterozygosity ranges from 4% in *Phlox drummondii* (LEVIN 1975a), to 6% in *Liatris cylindracea* (SCHAAL 1974), to 20% in both *Hymenopappus scabiosaeus* and *H. artemisiaefolius* (BABBEL and SELANDER 1974). Comparable values in the predominantly outbreeding *Lupinus subcarnosus* and *L. texensis* are 10% and 36%, respectively (*ibid.*).

Our data demonstrate the ability of permanent translocation heterozygotes to maintain genic heterozygosity in spite of enforced self-fertilization for more than 20 generations. This ability, rather than extraordinarily high levels of heterozygosity *per se*, distinguishes the ring-forming oenotheras from typical self-fertilizing plants. In plants of the latter type, heterozygosity rarely exceeds 5% even in the face of strong balancing selection (ALLARD, JAIN and WORKMAN 1968; ALLARD and KAHLER 1971, 1972). Comparisons between related oenotheras possessing different genetic systems are the most meaningful in assessing the selective basis of the structural hybridity, but such comparisons prove as equivocal as those preceding. Each ring-former is more heterozygous than the bivalent-former *Oe. hookeri*, in which no allozyme variation was detected in either wild populations or inbred strains (see Table 2). Presumably, each is also more heterozygous than the relict bivalent-former *Oe. grandiflora*; a single inbred strain of *Oe. grandiflora* available to us displays complete homozygosity for the majority allele at each locus in *Oe. biennis* (LEVY, STEINER and LEVIN 1975). However, despite enforced self-fertilization inbred strains of the relict bivalent-former *Oe. argillicola* maintain a mean heterozygosity of 8.0%. This value, although most likely a minimal estimate of heterozygosity in natural populations of *Oe. argillicola*, is more than twice that in *Oe. strigosa* and nearly equivalent to that in *Oe. biennis*. Moreover, differences in levels of polymorphism and heterozygosity between the ring-formers (see Table 2) appear to be correlated with historical

factors rather than due to disparate selective premiums on heterozygosity. Following CLELAND's phylogenetic sequence for the structural heterozygotes (shown in Figure 1) we find that the oldest, *Oe. parviflora*, has the greatest variability and heterozygosity and the youngest, *Oe. strigosa*, the least. This suggests that variability and heterozygosity, in part, are a function of the age of the taxa, i.e., they have accumulated over time. It appears that variability and heterozygosity are also functions of the disparity of the ancestral genomes of each structural heterozygote. From measures of genetic affinity between extant taxa (Tables 4 and 5) we may infer that CLELAND's Population 1 was rather divergent in allozyme constitution from the other ancestral stocks and that Populations 2, 3, 4 and 5 were relatively similar. Thus, *Oe. parviflora* may exhibit the greatest variability and heterozygosity among the structural heterozygotes primarily because its constituent genomes were the most divergent.

We view the origin of complex structural hybridity in Oenothera as the result of selection for increased fertility in areas of hybridization between taxa differing by several segmental arrangements. The greatest fertility and thus fitness would accrue to hybrids which had the lowest incidence of duplicate and deficient gametes. Originally, such hybrids might have had many small rings. But given sufficient time, additional translocations, and cross breeding, hybrids with a single ring and alternate disjunction would be produced and prevail. DARLINGTON (1958) writes, "In Oenothera hybridity for one interchange has favored hybridity for a second and a third. The ring of four has thus in rapid succession increased to include six, eight, and ten and finally all fourteen chromosomes. . . . In no other system, let us note, can a sequence of evolutionary events be specified so exactly." The view that complex structural hybridity is a means to escape sterility is consistent with the depauperate gene pools of each species and the *Oe. biennis* complex as a whole, the high level of genetic identity among taxa, and the large number of homozygous ring-forming strains.

The preceding hypothesis does not suggest that variability and heterozygosity owing to complex structural hybridity have been unimportant in the evolution of the ring-formers. Adaptation by genetic polymorphism may be reflected in clinal variation of gene frequencies. Such clines occur in each ring-former and, considering each polymorphic locus over all taxa, in seven of 48 north-south and east-west clines which might have been detected (Table 3). Three clines involve the *Got-1* locus, two associated with latitude and one with longitude.

Two different but not mutually exclusive explanations can be offered for the recurrent observations of clines. (1) Gradual clines in gene frequencies may be the product of migration between populations which are sufficiently isolated to permit divergence by random drift (WRIGHT 1951; KIMURA and MARUYAMA 1971). Considerable gene flow restriction in *Oe. biennis* is suggested by the fact that single populations in the Chicago region typically are uniform but different from other populations in the same area (LEVIN 1975b). However, it seems unlikely that selectively neutral polymorphisms and limited migration are the source of the clines because within a species there is no similarity in the pattern of geographic variation among loci. (2) Gradual clines in gene frequency may be

the product of disruptive selection across the range of species. This explanation would better fit the simultaneous perpendicular clines (at different loci) which are found within each species, since the pattern of selective pressures in space may vary among loci.

Recent theoretical and empirical investigations have suggested that the amount of polymorphism preserved in a species could be understood in terms of adaptation to patterns of spatial heterogeneity in the environment (LEVINS 1968; BEARDMORE 1970; MAYNARD SMITH 1970; SAMMETA and LEVINS 1970). However, it is difficult to interpret most of our data on the ring-formers in an ecological context. There is no relationship between the sizes of taxon ranges or the diversity of habitats they occupy and the number of alleles per polymorphic locus, mean heterozygosity, or the mean genetic identities between populations. The genetic identities of populations within taxa fail to decrease with increasing interpopulation distance even though ranges are extensive. Moreover, as mentioned previously, there is little evidence of allozyme variability within single populations. The sum total of our knowledge about members of the *Oe. biennis* complex suggests that adaptation to diverse habitats is accomplished primarily by developmental plasticity rather than genetic polymorphism. Members of the same seed parent are capable of surviving and reproducing under a broad spectrum of controlled culture regimes and exhibit over 1000-fold differences in biomass (KERSTER and LEVIN, unpublished observations). The great potential for the release of between-plant variation presumably is related to the ability of a single genotype to exploit diverse environments within and among populations. This adaptative strategy is consistent with that considered optimal for weedy plants (BRADSHAW 1965; LEVINS 1968), a life style well exemplified by the ring-forming oenotheras.

DOBZHANSKY (1951) hypothesized that the allelic contents of chromosome arrangements have been coadapted by selection so that different loci within gene arrangements produce a haploid genome that is physiologically balanced. Coadaptation may be manifest as genotype differences between different chromosome arrangements in the same population, or as genotype differences for a given chromosome arrangement derived from different localities. Evidence of genic differentiation both within and among various chromosome inversions has been obtained in Drosophila (PRAKASH and LEWONTIN 1968, 1971; PRAKASH and MERRITT 1972; PRAKASH and LEVITAN 1973). Questions now arise as to the genetic constitution of chromosome complexes of the ring-forming oenotheras. Does a single chromosome complex vary genetically from one locality to another? Do different chromosome complexes carry the same genotype of the 20 loci studied? We can address these questions from our data by comparing genic homozygotes from which gametic genotypes may be inferred. Seven of the *Oe. biennis* I strains we examined were homozygous in allozyme genotype and bore a common alpha or egg chromosome complex (segmental arrangement = 1·2, 3·4, 5·14, 7·10, 9·8, 11·12, 13·6; CLELAND 1972). The genotypes of these seven strains are listed in Table 7. Five of the seven homozygotes are different from the others. This demonstrates that a given chromosome arrangement may vary

TABLE 7

The genotypes of three segmental arrangements in Oe. biennis I

Arrangement and strain	Locus / Genotype *Pgm-1*	*Vlp*	*Got-1*	*Ltp-2*	*Pgi-1*	*Pgi-2*
α 1·2 3·4 5·14 7·10 9·8 11·12 13·6						
Hilltop, Indiana	b	b	b	b	b	a
Walkerton, Indiana	b	b	a	b	b	a
Cardiff Delta, Maryland	b	c	b	b	b	a
Cambridge I, Ohio	b	b	b	b	b	a
Lemoyne, Pennsylvania	b	c	a	b	b	a
Camp Perry, Virginia	b	c	a	b	b	a
Petersburg, Virginia	b	b	a	b	b	b
β 1·14 3·2 5·9 7·8 6·12 11·10 13·4						
Cardiff Delta, Maryland	b	c	b	b	b	a
Cambridge I, Ohio	b	b	b	b	b	a
Cambridge II, Ohio	b	b	a	b	b	a
β 1·4 3·9 5·2 7·8 6·12 11·10 13·14						
Newman, Illinois	b	b	b	b	b	a
Warsaw, Indiana	b	b	a	b	b	a
Lemoyne, Pennsylvania	b	c	a	b	b	a

genically from one locale to another and suggests that a given arrangement may store a considerable amount of variation. These interpretations are consistent with the morphological variation known to be associated with this arrangement (CLELAND, PREER and GECKLER 1950). The alpha complex in question is widely distributed across the range of *Oe. biennis* I and is judged to be the original alpha complex for this race (CLELAND 1972). Fifty-six of the 78 alpha biennis I complexes that have been fully analyzed by CLELAND and associates have this arrangement. Most of the other alpha complexes are one or two translocations removed.

The matter of whether different chromosome complexes carry the same genotypes is less clearly resolved from our data. The genotypes of two beta or sperm complexes, three strains each, have been determined from complete genic homozygotes, and are also presented in Table 7. It is evident that the beta complexes of Cambridge I and Newman, and the alpha complex of Hilltop and Cambridge I have the same genotype at polymorphic loci ($Pgm\text{-}1^b$, Vlp^b, $Got\text{-}1^b$, $Ltp\text{-}2^b$, $Pgi\text{-}1^b$, $Pgi\text{-}2^a$); they are identical at the remaining 14 loci. Whether gene frequencies are similar among alpha and beta complexes remains to be ascertained. The comparison of observed *versus* expected heterozygotes in the structural heterozygotes was an attempt to get at this and more general questions. This procedure is valid since the chromosome complexes of the egg and sperm are mutually exclusive. Unfortunately, the procedure is not very robust, and with the exception of some loci in *Oe. parviflora*, there was no evidence of gene frequency differences in the form of heterozygote excess.

The data obtained in this study are compatible with CLELAND's interpretation of phylogeny in the *Oe. biennis* complex (Figure 1), although in some instances we cannot differentiate between certain putative ancestors. The normalized genetic identities in excess of .85 indicate that *Oe. biennis*, *Oe. strigosa* and *Oe. hookeri* are very closely related. The one strain of *Oe. grandiflora* at our disposal has a genotype similar to that of *Oe. biennis* and its genetic identity to the aforementioned taxa also exceeds .85. It is informative to convert genetic identity to genetic distance (D), which reflects the accumulated number of amino acid differences per locus between closely related taxa (NEI 1972); $D = -\log_e I$. We find that the genetic distances among derivatives of Populations 2 through 5 are less than .16. These values are less than that typically found for taxon pairs which are only accorded subspecies status (NEI and CHAKRABORTY 1973). The genetic distance between *Oe. argillicola* and *Oe. biennis* is .67; it is representative of genetic distances between congeneric species in several genera. The disparity between *Oe. argillicola* and the remaining members of the complex is not surprising since *Oe. argillicola* shares certain morphological expressions with members of the closely related subgenus Raimannia which are absent in the subgenus Euoenothera except for one of the genomes of *Oe. parviflora* (CLELAND 1972).

Although in accord with CLELAND's phylogeny, we disagree with portions of his timetable. Recall that CLELAND (1972) favors the view that the *Oe. biennis* complex is the product of five ancestral populations which arose successively from a Mexican-Central American nucleus during different periods of Pleistocene glaciation. CLELAND's timetable places the divergence of *Oe. argillicola* lineage from the ancestral nucleus at about 1,000,000 years, with subsequent divergences placed more or less equidistant in time. Crude estimates of the divergence times of the taxa in the *Oe. biennis* complex may be obtained following the formulae and approximations offered by NEI (1971, 1974). Assuming the rate of gene substitution to be the same for each enzyme locus, the expected number of amino acid per protein differences between species is estimated by $D = 2\, cn_T\lambda_a\, t$, where c is the proportion of amino acid substitutions that can be detected by electrophoresis, n_T is the number of codons concerned with the synthesis of a protein, λ_a is the rate of amino acid substitution per site per year, and t is the period of time since a pair of taxa become isolated. Then $t = D/2\, cn_T\lambda_a$. Although nothing is known about n_T or λ_a in Oenothera, values obtained from other organisms may be used for a rough approximation. NEI chooses $n_T = 800$ and $\lambda_a = = 2.1 \times 10^{-9}$ as plausible numbers. The former is based upon published accounts of protein molecular weights, and the latter on the mean values of λ_a for 13 different proteins from vertebrates. NEI's estimate of c is .3, and is based upon genetic code tables and the proportion of single base changes which give rise to amino acid substitutions resulting in an altered net charge of protein (given pH $6.00 \sim 8.33$). Assuming that the aforementioned values are the same for all proteins, the times of divergence of the *Oe. argillicola* lineage from the others is roughly as follows: *Oe. hookeri*, 8.13×10^5 years; *Oe. strigosa*, 6.84×10^5 years; *Oe. biennis* (all races) 6.65×10^5 years. We surmise that the divergence time of *Oe. grandiflora* would be similar to that of *Oe. biennis*. Among the taxa which are quite similar,

the divergence time of *Oe. hookeri* and *Oe. biennis* is roughly 1.48×10^5 years, while the time for *Oe. hookeri* and *Oe. strigosa* is roughly 1.29×10^5 years. *Oenothera biennis* and *Oe. strigosa* seem to have diverged about 19,000 years ago. In *Oe. biennis* and *Oe. parviflora*, racial divergence occurred roughly 10,000 years ago, or during the last glacial period. The estimates of divergence time suggest that *Oe. argillicola* split off the remainder of the Euoenothera core near the beginning of the Pleistocene as CLELAND surmised. We surmise that subsequent evolution leading to contemporary taxa did not occur for several hundred thousand years, with the major diversification ensuing during the past 150,000 years rather than during widely spaced glacial periods.

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LITERATURE CITED

ALLARD, R. W. and A. L. KAHLER, 1971 Allozyme polymorphisms in plant populations. Stadler Symposia **3**: 9–24. Univ. Missouri Press, Columbia. ——, 1972 Patterns of molecular variation in plant populations. Proc. Sixth Berkeley Symposium on Mathematical Statistics and Probability. **5**: 237–254.

ALLARD, R. W., S. K. JAIN and P. L. WORKMAN, 1968 The genetics of inbreeding populations. Advan. Genet. **14**: 55–131.

BABBEL, G. R. and R. K. SELANDER, 1974 Genetic variability in edaphically restricted and widespread plant species. Evolution **28**: 619–630.

BEARDMORE, J., 1970 Ecological factors and variability in gene pools. pp. 299–314. In: *Essays in evolution and genetics in honor of Theodosius Dobzhansky*. Edited by M. K. HECHT and W. C. STEERE. Appleton-Century-Crofts, New York.

BRADSHAW, A. D., 1965 Evolutionary significance of phenotypic plasticity in plants. Advan. Genet. **13**: 115–155.

BHADURI, P. N., 1942 Further cytological investigations in the genus *Gaura*. Ann. Botany **6**: 229–244.

CLELAND, R. E., 1936 Some aspects of the cytogenetics of *Oenothera*. Bot. Rev. **2**: 316–348. ——, 1944 The problem of species in Oenothera. Am. Naturalist **78**: 5–28. ——, 1972 *Oenothera: cytogenetics and evolution*. Academic Press, New York.

CLELAND, R. E., L. B. PREER and L. H. GECKLER, 1950 The nature and relationships of taxonomic entities in the North American Euoenotheras. In: *Studies in Oenothera cytogenetics and phylogeny*. Edited by R. CLELAND. Ind. Univ. Publ. Sci. Ser. **16**: 218–254.

DARLINGTON, C. D., 1937 *Recent advances in cytology*. Churchill, London. ——, 1958 *The evolution of genetic systems*. 2nd Ed. Oliver & Boyd, London.

DELORENZO, R. J. and F. H. RUDDLE, 1970 Glutamate-oxalate-transaminase (GOT) genetics in *Mus musculus*: linkage, polymorphism, and phenotypes of the GOT-2 and GOT-1 loci. Biochem. Genet. **4**: 259–273.

DOBZHANSKY, TH., 1951 *Genetics and the origin of species*. 3rd Ed. Columbia Univ. Press, New York.

HEDRICK, P. W., 1971 A new approach to measuring genetic identity. Evolution **25**: 276–280.

HOAR, C. S., 1931 Meiosis in *Hypericum punctatum*. Botan. Gaz. **92**: 396–406.

JAMES, S. H., 1965 Complex hybridity in *Isotoma petraea*. I. The occurrence of interchange heterozygosity, autogamy and a balanced lethal system. Heredity **20**: 341–353. ——, 1970 Complex hybridity in *Isotoma petraea*. II. Components and operations of a possible evolutionary mechanism. Heredity **25**: 53–78.

KIMURA, M., and T. MARUYAMA, 1971 Pattern of neutral polymorphism in a geographically structured population. Genet. Res. **18**: 125–131.

LEVIN, D. A., 1975a Interspecific hybridization, heterozygosity and gene exchange in *Phlox*. Evolution (In press.) ——, 1975b Genic heterozygosity and protein polymorphism among local populations of *Oenothera biennis*. Genetics (this issue).

LEVIN, D. A. and W. L. CREPET, 1973 Genetic variation in *Lycopodium lucidulum*: a phylogenetic relict. Evolution **27**: 622–632.

LEVIN, D. A., G. P. HOWLAND and E. STEINER, 1972 Protein polymorphism and genic heterozygosity in a population of the permanent translocation heterozygote, *Oenothera biennis*. Proc. Natl. Acad. Sci. U.S. **69**: 1475–1477.

LEVINS, R., 1968 *Evolution in changing environments*. Princeton Univ. Press, Princeton.

LEVY, M., E. STEINER and D. A. LEVIN, 1975 Allozyme genetics in permanent translocation heterozygotes of the genus Oenothera. Biochem. Genet. (In press.)

LEWIS, H. and J. SZWEYKOWSKI, 1964 The genus *Gayophytum* (Onagraceae). Brittonia **16**: 343–392.

LIN, Y. J., 1973 Position and frequency of adjacent distribution of meiotic chromosomes in *Rhoeo spathacea*. Am. J. Botany **60**: 658–690.

MARSHALL, D. R. and R. W. ALLARD, 1970 Isozyme polymorphisms in natural populations of *Avena fatua* and *A. barbata*. Heredity **25**: 373–382.

MAYNARD SMITH, J., 1970 Genetic polymorphism in a varied environment. Am. Naturalist **104**: 487–490.

NEI, M., 1971 Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. Am. Naturalist **105**: 385–398. ——, 1972 Genetic distance between populations. Am. Naturalist **106**: 283–292. ——, 1974 The theory and estimation of genetic distances. pp. 45–54. In: *Genetic structure of populations*. Edited by N. E. MORTON. Univ. Hawaii Press, Honolulu.

NEI, M. and R. CHAKRABORTY, 1973 Genetic distance and electrophoretic identity of proteins between taxa. J. Molec. Evol. **2**: 323–328.

PRAKASH, S. and M. LEVITAN, 1973 Association of alleles of the esterase-1 locus with gene arrangements of the left arm of the second chromosome in *Drosophila robusta*. Genetics **75**: 371–379.

PRAKASH, S. and R. C. LEWONTIN, 1968 A molecular approach to the study of genic heterozygosity in natural populations. III. Direct evidence of coadaptation in gene arrangements of Drosophila. Proc. Natl. Acad. Sci. U.S. **59**: 398–405. ——, 1971 A molecular approach to the study of genic heterozygosity in natural populations. V. Further direct evidence of coadaptation in inversions of Drosophila. Genetics **69**: 405–408.

PRAKASH, S. and R. B. MERRITT, 1972 Direct evidence of genic differentiation between sex ratio and standard gene arrangements of *X* chromosome in *Drosophila pseudoobscura*. Genetics **72**: 169–175.

RAVEN, P. H. and D. P. GREGORY, 1972 Observations of meiotic chromosomes in *Gaura* (Onagraceae). Brittonia **24**: 71–86.

RUDDLE, F. H. and E. NICHOLS, 1971 Starch gel electrophoretic phenotypes of mouse and human somatic cell hybrids and mouse isozyme polymorphisms. In Vitro **7**: 120–131.

SAMMETA, K. P. V. and R. LEVINS, 1970 Genetics and ecology. Ann. Rev. Genet. **4**: 469–488.

SAX, K., 1931 Chromosome ring formation in *Rhoeo discolor*. Cytologia **3**: 36–53. ——, 1935 Chromosome structure in the meiotic chromosomes of *Rhoeo discolor* Hance. J. Arnold Arbor. **16**: 216–224.

SCHAAL, B. A., 1974 Genetic population structure and selection in *Liatris cylindracea*. Ph.D. dissertation. Yale University, New Haven, Connecticut.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON and J. B. GENTRY, 1971 Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics. VI. Univ. Texas Publ. **7103**: 49–90.

SHAW, C. R. and R. PRASAD, 1970 Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. **4**: 297–320.

SOKAL, R. R. and F. J. ROHLF, 1969 *Biometry*. Freeman, San Francisco.

STEBBINS, G. L., 1950 *Variation and evolution in plants*. Columbia Univ. Press, New York. ——, 1971 *Chromosomal evolution in higher plants*. Addison Wesley, Reading, Mass.

STEBBINS, G. L. and S. ELLERTON, 1939 Structural hybridity in *Paeonia californica* and *P. brownii*. J. Genet. **38**: 1–36.

WALTERS, J. L., 1942 Distribution of structural hybrids in *Paeonia californica*. Am. J. Botany **29**: 270–275.

WALTERS, M. S. and D. U. GERSTEL, 1948 A tetraploid *Rhoeo discolor*. Am. J. Botany **35**: 141.

WRIGHT, S., 1951 The genetical structure of populations. Ann. Eugen. **15**: 323–354.

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