GENETIC AND REPRODUCTIVE DIFFERENTIATION OF THE SUBSPECIES, *DROSOPHILA EQUINOXIALIS CARIBBENSIS*[1]

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Sibling species are species morphologically so similar as to be practically indistinguishable from each other by inspection of their structural features. The *Drosophila willistoni* group consists of at least twelve species, six of which are siblings. Two of the sibling species, *D. pavlovskiana* Dobzhansky and Kastritis, and *D. insularis* Dobzhansky, are narrow endemics; the former in Guyana and the latter on some islands of the Lesser Antilles. The other four species, *D. willistoni* Sturtevant, *D. equinoxialis* Dobzhansky, *D. paulistorum* Dobzhansky and Pavan, and *D. tropicalis* Burla and de Canba, have wide and largely overlapping geographic distributions. The four species are sympatric through Central America and the northern half of continental South America; two or three of them are sympatric in the southern half of Mexico, the larger Caribbean islands, Peru, Bolivia, and the southern half of Brazil (Spassky et al., 1971).

We have studied for several years genetic variation in the sibling species of the *D. willistoni* group, using techniques of gel electrophoresis and enzyme assay. *D. equinoxialis* consists of two subspecies, *D. e. equinoxialis* and *D. e. caribbensis* (Ayala, 1973). In this paper we report results of the study of reproductive isolation between these two subspecies, and we compare genetic variation within and between the subspecies. Ayala and Powell (1972) have shown that allozymes, i.e., enzymes coded for by alleles at the same gene locus, can be used in *Drosophila* as diagnostic characters of sibling species. We show now that they can also be used as subspecies diagnostic characters. Substantial genetic differentiation exists between the two subspecies of *D. equinoxialis*.

GEOGRAPHIC DISTRIBUTION AND MATERIALS

Drosophila equinoxialis was described by Dobzhansky (1946) from Tefe, State of Amazonas, Brazil. The known distribution of *D. equinoxialis* extends from central Mexico through Central America, the Greater Antilles, and the northern half of continental South America, that is down to approximately 10°L.S. It has not been found south of the Amazon basin, nor in the Caribbean east of Puerto Rico, in spite of extensive collections made in several of the Lesser Antilles.

On June 15, 1969, Professor Hampton L. Carson collected several *D. equinoxialis* flies in the U.S.D.A. Experimental Station in Mayagüez, Puerto Rico. We found that progenies of these flies when crossed to *D. equinoxialis* strains from continental South America yield fertile females and sterile males. In 1970, 1971 and 1972, collections were made in Costa Rica, Puerto Rico and Hispaniola. Strains from any one of these locations produce fertile males and females when intercrossed with each other; however, they produce fertile females but sterile males when crossed with strains collected in eastern Panama or in continental South America. *D. equinoxialis* is thus subdivided into two subspecies (Ayala, 1973): *D. e. equinoxialis* in eastern Pan-

[1] This paper is dedicated to PROFESSOR ERNST MAYR in recognition of his important contributions to modern evolutionary theory on the occasion of his 70th birthday. This paper is number VII in a series entitled "Enzyme variability in the *Drosophila willistoni* group."

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ama, in continental South America and Trinidad; and *D. e. caribbensis* in Puerto Rico, Hispaniola and Costa Rica. Whether the *caribbensis* subspecies extends north of Costa Rica through Central America and southern Mexico, and in Cuba and Jamaica, remains conjectural.

The materials used in the present study are as follows.

D. e. caribbensis.—(1) Tortuguero, Limón, Costa Rica—four collecting sites separated by 1–3 km from each other, collected by Dr. David Ehrenfeld and one of us (JGE) in September 1970 and July 1971. (2) Santiago, Dominican Republic—one collection about 10 km northeast of the city of Santiago, in the foothills of the Cordillera Septentrional, collected by one of us (FJA) in March 1972. (3) Santo Domingo, Dominican Republic—one collection about 25 km north of the city of Santo Domingo, east of the main road between that city and Santiago, collected by FJA in March 1972. (4) Mayagüez, Puerto Rico, in the U.S.D.A. Experimental Station—two collections, one by Professor H. L. Carson in June 1969, and the second by FJA in February 1972. (5) Barranquitas, Puerto Rico—one collection made in February 1972 by FJA in a small forest (less than 0.01 sq. km) within the grounds of the home of Dr. Roberto Aponte, M.D., on the Cordillera Central. (6) Yunque, Puerto Rico—several collections made in February 1972 by Dr. David L. Bruck, several graduate students of the University of Puerto Rico, Rio Piedras, and FJA, on the place called La Mina in the Luquillo Forest, Sierra de Luquillo.

D. e. equinoxialis. (1) Jaqué, Panamá. (2) Teresita, Chocó, Colombia. (3) Burguillo, southeast of Caracas, Venezuela. (4) Puerto Ayacucho, Venezuela. (5) Rancho Grande, Venezuela. Strains from these localities were used for studies of reproductive isolation. Information concerning these localities can be found in Spassky et al. (1971). Additional localities were sampled for electrophoretic studies; these localities are listed in Ayala et al. (1972*a*).

Sexual Isolation

Two different techniques are used to study sexual isolation. First, a "no-choice" method. Groups of 10 virgin females from one strain are placed together with about 10 males of the same or of a different strain in half-pint culture bottles with standard medium. The cultures are kept at 25 ± 0.5C. Five days later the females and males are transferred, without etherization, to fresh culture bottles. After five more days the surviving females are dissected, and their reproductive tracts examined under a microscope for the presence or absence of sperm. The results are summarized in Table 1, which shows the percentages of females inseminated in intrasubspecific or intersubspecific crosses between the strains. The number of the females tested in each case is omitted, but the total numbers of females of each strain dissected are given in the last column of the table. In all, 1685 females were dissected in this experiment.

Intercrosses within or between the subspecies occur quite easily. The crosses involving males and females of only *D. e. equinoxialis* are almost always 100% successful. When *D. e. caribbensis* flies are used either as females, or as males, or both, the proportion of females inseminated is somewhat less than 100%; however, the proportions of females inseminated are not lower in intersubspecific crosses than in intrasubspecific ones. The *averages* of the percent females inseminated are 93.3 and 91.3 for the intersubspecific and intrasubspecific crosses, respectively, involving *D. e. caribbensis*. The *actual* percentage of females inseminated is 89.3 (994 out of 1113 females dissected) in intersubspecific crosses, but 89.7 (287 out of 320 females dissected) in intrasubspecific crosses involving *D. e. caribbensis*.

A joint index of sexual isolation, K, for these "no-choice" experiments can be calculated as the percentage of females inseminated by males of the same strain minus the percentage of females insemi-

TABLE 1. *Sexual preferences among strains of* Drosophila equinoxialis equinoxialis *and* D. e. caribbensis. *The figures given represent the percentage of females inseminated by the corresponding males. The total number of females dissected of both subspecies is 1685.*

♀♀ \ ♂♂	*D. e. equinoxialis*						*D. e. caribbensis*			♀♀ dissected
	Jaque	Teresita	Burguillo	P. Ayacucho	Rancho Grande	Total	Tortuguero	P. Rico	Total	
D. e. equinoxialis										
Jaque	100	100				100	94	98	95	164
Teresita	100	100	100		100	100	97	100	99	155
Burguillo		100		100		100	96	84	93	203
P. Ayacucho			100			100	97		97	135
Rancho Grande		100				100	100	100	100	66
Total	100	100	100	100	100	100	96	96	96	723
D. e. caribbensis										
Tortuguero	83	93	81	67		78	82	84	83	625
Puerto Rico	96	100	100		100	99	99	100	99	337
Total	89	96	83	67	100	84	92	87	90	962

nated by males of a different strain, divided by the sum of both percentages (Ayala, 1965). The value of K can range from +1 (complete sexual isolation) to −1 (all matings heterogametic); its value is zero when matings occur at random. We have calculated the value of K for all pairs of strains for which the appropriate information is available in Table 1; none of the K's is statistically significantly different from zero. The mean value of all K's is 0.002 ± 0.005, not significantly different from zero. (Standard error calculated from the variance between the K's). If the data for all strains of a given subspecies are pooled, the joint index of sexual isolation between the subspecies is $K = 0.014 \pm 0.017$, again not significantly different from zero. (Standard deviation calculated from the binomial variance). These "no-choice" experiments give no evidence of sexual isolation between *D. e. equinoxialis* and *D. e. caribbensis*, nor between strains of any one subspecies.

We have used a second method to study sexual isolation—the "observation chamber" of Elens (1958). This technique has the advantage over the no-choice method of permitting four kinds of matings in a single chamber, that is, $♀A \times ♂A$, $♀B \times ♂B$, $♀A \times ♂B$, and $♀B \times ♂A$, where A and B symbolize two different strains. 12 virgin females and 12 males of each one of two strains, separately aged for at least five days, are introduced into a chamber where matings can be directly observed. To make the flies distinguishable, the distal margins of the wing are clipped in the flies of one strain; this marking is rotated from strain to strain in replicate experiments. The flies in a chamber are continuously observed, and the matings recorded, for about three hours. Usually, most matings occur during the first hour; very few, if any, during the third hour of observation.

The results are summarized in Table 2, where each combination of two strains has been replicated four times. Sexual isolation is measured in the observation chambers by

$$I = (x_{AA} + x_{BB} - x_{AB} - x_{BA})/N \quad (1)$$

where x_{AA}, x_{BB}, x_{AB} and x_{BA} stand for the four types of matings, $♀A \times ♂A$, $♀B \times ♂B$, $♀A \times ♂B$, and $♀B \times ♂A$, respectively; and $N = x_{AA} + x_{BB} + x_{AB} + x_{BA}$. The value of I can range from +1 to −1. It is zero when matings occur at random.

The variance of I is (Malogolowkin-Cohen et al., 1965)

$$\sigma_I^2 = (1 - I^2)/N \quad (2)$$

TABLE 2. *Matings between strains of* Drosophila equinoxialis equinoxialis *and* D. e. caribbensis *in observation chambers.* I = *index of sexual isolation.*

A	B	♀A × ♂A	♀B × ♂B	♀A × ♂B	♀B × ♂A	Total matings	% homogametic matings	*I*
Jaque × Burguillo		21	13	10	19	63	54.0	.079 ± .126
Jaque × P. Ayacucho		26	10	11	15	62	58.1	.161 ± .125
Burguillo × P. Ayacucho		27	22	7	23	79	62.0	.241 ± .109*
Tortuguero × P. Rico		28	11	6	17	62	62.9	.258 ± .123*
Total intrasubspecific matings		102	56	34	74	266	59.4	.185 ± .041*
Jaque × Tortuguero		17	28	31	9	85	52.0	.059 ± .108
Burguillo × Tortuguero		13	37	25	4	79	63.3	.266 ± .108*
P. Ayacucho × Tortuguero		11	33	24	1	69	63.8	.275 ± .116*
Jaque × P. Rico		12	30	27	7	76	55.3	.105 ± .114
Burguillo × P. Rico		9	28	22	12	71	52.1	.042 ± .119
P. Ayacucho × P. Rico		17	26	19	13	75	57.3	.147 ± .114
Teresita × P. Rico		12	12	15	14	53	45.3	.094 ± .137
Total intersubspecific matings		91	194	163	60	508	56.1	.114 ± .049
Grand total		193	250	197	134	774	57.2	.140 ± .035*

* Statistically significant, $P < 0.05$.

The indices of sexual isolation with their standard deviations, obtained according to (2), are given in the last column of Table 2. Statistical significance is calculated using Student's *t*. There is evidence of incipient sexual isolation between geographic strains of *D. equinoxialis*. In 10 out of 11 combinations, the value of *I* is positive; in four cases it is significantly greater than zero. The mean value of *I* for all 11 combinations (last row of Table 2) is 0.140 ± 0.035 which is significantly positive, $P < 0.01$. (The standard error has been calculated from the variance among the *I*'s). However, sexual isolation is not greater between strains of different subspecies ($I = 0.114 \pm 0.049$) than between strains of the same subspecies ($I = 0.185 \pm 0.041$).

Each combination of two strains as given in Table 2 was replicated four times. We have calculated the index of sexual isolation according to (1) for *each individual chamber*, and then have obtained the mean for each combination of strains with a standard error calculated from the variance among replicates. This procedure yields results which are virtually identical with those obtained using the pooled data as in Table 2. The means and standard errors of *I* are 0.178 ± 0.038 for intrasubspecific, 0.112 ± 0.049 for intersubspecific, and 0.136 ± 0.034 for all combinations.

In conclusion, we have demonstrated incipient sexual isolation between geographic strains of *D. equinoxialis* using mating chambers, while no such evidence appears in the "no-choice" tests. It seems that the mating chamber is a more sensitive method to study sexual isolation than the no-choice technique.

HYBRID STERILITY

The fertility of hybrid progenies is tested as follows. Groups of 10 virgin females of one strain and 10 males of a different strain are placed together in a culture bottle; after 10 days these flies are discarded. When progenies are produced, at least 20 male and 20 female F_1 flies are placed in a fresh culture bottle. If F_2 progenies are produced, both male and female F_1 hybrids are scored as fertile. If F_2 progenies are not produced, groups of

TABLE 3. *Outcome of crosses between strains of* Drosophila equinoxialis equinoxialis *and* D. e. caribbensis. *F = fertile F_1 hybrids; S = hybrids produced but F_1 males sterile; SF = some F_1 hybrid males sterile, some fertile.*

♀♀ \ ♂♂	*D. e. equinoxialis*				*D. e. caribbensis*	
	Jaque	Teresita	Burguillo	Rancho Grande	Tortuguero	P. Rico
D. e. equinoxialis						
Jaque	F	F	F	—	S	S
Teresita	F	F	—	F	S	S
Burguillo	F	—	F	—	SF	SF
Rancho Grande	F	F	—	F	—	SF
D. e. caribbensis						
Tortuguero	S	S	SF	—	F	F
P. Rico	S	S	S	SF	F	F

at least 10 F_1 females are placed in a culture with at least 10 males of one or the other parental strain. If progenies are produced by these backcrosses, the F_1 hybrid females are scored as fertile and the F_1 hybrid males as sterile.

The main results are clear (Table 3). Crosses between any two strains produce abundant F_1 progenies. Crosses between strains of the same subspecies also produce abundant F_2 progenies. Crosses between strains of different subspecies generally do not produce F_2 progenies. The F_1 females are always fertile in the intersubspecific crosses, but the F_1 males are generally sterile. Crosses between subspecies involving the Burguillo or the Rancho Grande strains occasionally produce some F_1 fertile males.

The fertility of F_1 males in some intersubspecific crosses conceivably may only be apparent, not real; i.e., it is possible that one or several parental females were not virgin in a given cross, and therefore that some of the F_1 flies are not hybrid at all. This possibility cannot be completely ruled out but it is unlikely. All crosses reported in Table 3 were repeated in at least three different sets, each set prepared several months after the previous one. The third set was prepared by a different worker than the two other sets. Within a set, each cross was replicated at least twice, often three to five times. The crosses recorded as occasionally yielding fertile F_1 males (*SF* in the Table), produced F_2 progenies in at least two different sets made by two different workers. At least 20 F_1 males were used in each test; since F_2 progenies appeared only occasionally it appears that intersubspecific crosses produce fertile F_1 males with a very low frequency. In any case, our results show that crosses between the subspecies yield, at least in most cases, sterile F_1 males.

GENETIC VARIATION IN *D. e. caribbensis*

We have studied genetic variation at 27 loci coding for enzymes in six natural populations of *D. e. caribbensis*; one in Costa Rica (Tortuguero), two in Hispaniola (Santiago and Santo Domingo), and three in Puerto Rico (Mayagüez, Barranquitas, and Yunque). We have used techniques for gel electrophoresis and enzyme assay which are described elsewhere (Ayala et al., 1972*a*,*b*). Two additional enzymes have been assayed in the present study. *Aldehyde oxidase* (*Ao*): Electrostarch; gel and electrode buffer: 87 mM Tris, 8.7 mM boric acid, and 1mM EDTA, pH 9.0; run for 6 hr at 25 v/cm; stain: 20 mg NBT, 25 mg NAD, 10 mg EDTA, 1.0 ml benzaldehyde, 5 mg PMS, in 100 ml 0.05 Tris-HCl buffer, pH 8.6; incubate in the dark at room temperature. *Xanthine dehydrogenase* (*Xdh*): Electrostarch; gel

TABLE 4. *Genetic variation at 27 loci in natural populations of* Drosophila equinoxialis.

		D. e. caribbensis						
Gene	Alleles	Tortuguero	Santiago	Santo Domingo	Mayagüez	Barranquitas	Yunque	*D. e. equinoxialis*
Lap-5	Sample size	662	188	194	572	612	20	2774
	105	.000	.000	.000	.000	.000	.000	.205
	106	.169	.011	.000	.010	.010	.000	.000
	107	.784	.739	.753	.778	.747	.750	.712
	109	.050	.245	.247	.206	.234	.250	.075
	Heterozygotes	.355	.394	.372	.352	.387	.375	.486 ± .023
Est-2	Sample size	322	192	200	326	598	20	834
	98	.025	.052	.045	.031	.050	.050	.014
	100	.320	.047	.055	.068	.033	.150	.032
	102	.630	.885	.855	.890	.860	.650	.939
	Heterozygotes	.486	.212	.262	.202	.255	.540	.126 ± .019
Est-3	Sample size	350	128	60	306	232	10	110
	98	.014	.008	.017	.016	.009	.100	.473
	100	.794	.977	.967	.977	.978	.900	.400
	102	.174	.016	.017	.006	.009	.000	.118
	Heterozygotes	.339	.045	.064	.045	.043	.180	.566 ± .029
Est-4	Sample size	712	192	206	392	644	20	2682
	98	.902	.995	.976	.954	.992	.950	.150
	100	.087	.000	.010	.028	.005	.000	.769
	102	.000	.000	.000	.003	.000	.050	.081
	Heterozygotes	.179	.010	.047	.088	.016	.095	.373 ± .045
Est-5	Sample size	672	192	198	544	620	20	2696
	95	.007	.000	.005	.004	.000	.000	.033
	100	.545	.411	.338	.381	.348	.350	.940
	105	.440	.588	.626	.601	.645	.600	.024
	110	.006	.000	.030	.015	.006	.050	.000
	Heterozygotes	.509	.485	.493	.494	.463	.515	.090 ± .011
Est-6	Sample size	266	–	–	22	–	–	2756
	100	.014	–	–	.000	–	–	.014
	102	.056	–	–	.000	–	–	.007
	104	.716	–	–	.545	–	–	.843
	106	.094	–	–	.046	–	–	.023
	108	.117	–	–	.409	–	–	.111
	Heterozygotes	.459	–	–	.533	–	–	.256 ± .027
Aph-1	Sample size	215	84	72	346	166	4	1913
	100	.051	.000	.028	.043	.012	.000	.020
	102	.902	.976	.972	.922	.952	1.000	.919
	104	.042	.024	.000	.035	.024	.000	.057
	Heterozygotes	.185	.047	.054	.147	.093	.000	.133 ± .023
Acph-1[2]	Sample size	452	198	218	526	648	20	702
	88	.894	.545	.537	.557	.574	.800	.000
	94	.064	.045	.050	.010	.002	.050	.013
	100	.000	.207	.133	.171	.114	.000	.172
	102	.000	.005	.046	.000	.000	.000	.000
	104	.018	.030	.037	.013	.012	.100	.811
	106	.000	.156	.179	.236	.262	.000	.000
	108	.007	.010	.005	.006	.017	.000	.004
	Heterozygotes	.219	.633	.656	.604	.588	.345	.308 ± .010
Ald	Sample size	–	110	116	232	242	16	66
	98	–	.000	.009	.009	.000	.000	.061

[1] Some rare alleles have been omitted from the table.
[2] Locus diagnostic of the subspecies.

TABLE 4. *Continued.*

Gene	Alleles	Tortuguero	Santiago	*D. e. caribbensis* Santo Domingo	Mayagüez	Barranquitas	Yunque	*D. e. equinoxialis*
	100	–	.700	.629	.703	.831	.500	.833
	102	–	.291	.345	.280	.157	.500	.091
	Heterozygotes	–	.425	.485	.428	.285	.500	.297 ± .007
Adh	Sample size	302	198	224	418	624	20	1860
	98	.089	.000	.000	.002	.000	.000	.135
	100	.904	.995	1.000	.993	.998	1.000	.861
	Heterozygotes	.167	.010	.000	.014	.004	.000	.232 ± .032
Mdh-2[2]	Sample size	616	114	154	272	400	16	1900
	94	.002	.000	.000	.000	.000	.000	.994
	106	.994	1.000	.994	.960	.998	1.000	.000
	Heterozygotes	.021	.000	.012	.078	.004	.000	.010 ± .004
αGpdh	Sample size	638	198	224	630	658	20	1888
	100	.987	.995	1.000	.998	1.000	1.000	.983
	Heterozygotes	.021	.010	.000	.003	.000	.000	.027 ± .009
Idh	Sample size	264	198	226	306	610	20	822
	100	.955	1.000	.987	1.000	.998	1.000	.960
	104	.034	.000	.000	.000	.002	.000	.038
	Heterozygotes	.088	.000	.026	.000	.004	.000	.088 ± .023
G3pdh[2]	Sample size	–	110	120	282	242	–	32
	92	–	.027	.000	.021	.021	–	.000
	96	–	.954	.958	.933	.930	–	.063
	100	–	.009	.000	.043	.037	–	.906
	Heterozygotes	–	.089	.081	.127	.133		.196
Odh-1	Sample size	245	114	154	132	370	16	508
	96	.008	.009	.006	.000	.000	.000	.104
	100	.971	.965	.994	1.000	.992	.938	.827
	104	.004	.026	.000	.000	.005	.062	.057
	Heterozygotes	.074	.068	.012	.000	.016	.116	.293 ± .073
Me-1	Sample size	370	114	154	294	386	16	734
	100	.022	.000	.000	.000	.005	.000	.007
	104	.976	1.000	1.000	.996	.995	1.000	.990
	Heterozygotes	.047	.000	.000	.007	.010	.000	.025 ± .010
Me-2	Sample size	50	114	154	284	354	16	292
	96	.040	.018	.020	.018	.023	.000	.137
	100	.840	.912	.870	.845	.895	.750	.801
	104	.060	.061	.104	.134	.076	.250	.062
	Heterozygotes	.287	.164	.232	.268	.193	.375	.308 ± .058
Xdh	Sample size	–	198	226	572	610	20	–
	98	–	.000	.013	.002	.010	.050	–
	99	–	.020	.018	.019	.048	.000	–
	100	–	.475	.571	.367	.546	.400	–
	101	–	.485	.385	.505	.380	.400	–
	102	–	.020	.009	.107	.016	.150	–
	Heterozygotes	–	.538	.525	.598	.555	.655	–
Ao-1	Sample size	–	98	106	86	166	–	–
	98	–	.000	.038	.000	.000	–	–
	100	–	.000	.840	.000	.000	–	–
	103	–	.051	.076	.035	.006	–	–
	104	–	.000	.000	.017	.024	–	–
	105	–	.908	.038	.919	.904	–	–
	106	–	.031	.000	.035	.006	–	–
	Heterozygotes	–	.172	.277	.153	.179	–	–

TABLE 4. *Continued.*

Gene	Alleles	*D. e. caribbensis*							
		Tortuguero	Santiago	Domingo Santo	Mayagüez	Barranquitas	Yunque	*D. e. equinoxialis*	
To	Sample size	530	156	190	476	513	18	1347	
	98	.000	.000	.000	.000	.000	.000	.034	
	100	.996	1.000	1.000	.996	.998	1.000	.956	
	Heterozygotes	.003	.000	.000	.008	.004	.000	.131	± .046
Tpi-2	Sample size	318	114	152	284	402	16	550	
	106	.975	.982	.987	1.000	1.000	1.000	.978	
	Heterozygotes	.064	.036	.026	.000	.000	.000	.037	± .013
Pgm-1	Sample size	6	114	152	302	400	16	524	
	96	.000	.009	.000	.000	.002	.000	.011	
	100	.667	.053	.105	.079	.030	.062	.353	
	104	.333	.930	.895	.904	.962	.938	.622	
	108	.000	.009	.000	.010	.005	.000	.011	
	Heterozygotes	.444	.132	.188	.176	.074	.116	.444	± .034
Adk-1	Sample size	212	114	154	208	278	16	658	
	100	.420	.070	.039	.303	.086	.438	.386	
	106	.566	.702	.818	.654	.791	.562	.547	
	112	.014	.219	.136	.043	.108	.000	.059	
	Heterozygotes	.491	.454	.311	.479	.355	.492	.474	± .034
Adk-2	Sample size	74	114	152	284	402	16	576	
	100	.135	.000	.000	.004	.000	.000	.038	
	104	.851	.982	1.000	.961	.988	.938	.941	
	108	.014	.018	.000	.028	.012	.062	.007	
	Heterozygotes	.257	.035	.000	.076	.024	.116	.096	± .052
Hk-1[a]	Sample size	22	114	154	282	400	16	428	
	96	.955	.982	.980	.975	.990	.938	.082	
	100	.045	.000	.013	.011	.005	.062	.914	
	Heterozygotes	.087	.035	.039	.049	.020	.057	.151	± .028
Hk-2	Sample size	174	114	154	284	400	16	662	
	96	.006	.009	.000	.014	.000	.000	.014	
	100	.862	.974	.812	.958	.958	1.000	.920	
	104	.063	.009	.006	.021	.015	.000	.044	
	108	.069	.009	.182	.007	.028	.000	.000	
	Heterozygotes	.296	.051	.308	.082	.081	.000	.146	± .041
Hk-3	Sample size	174	114	154	296	400	16	676	
	96	.011	.009	.026	.007	.005	.000	.007	
	100	.971	.974	.961	.970	.992	1.000	.954	
	104	.017	.009	.013	.024	.002	.000	.036	
	Heterozygotes	.032	.051	.076	.059	.016	.000	.122	± .038

and electrode buffer as for acetaldehyde oxidase; run for 6 hr at 25 v/cm; stain: 100 mg hypoxanthine into 100 ml 0.05 M Tris-HCl buffer, pH 7.5, heat to boiling while stirring to get hypoxanthine into solution, cool to room temperature and add 20 mg NBT, 25 mg NAD, 15 mg KCL, and 5 mg PMS; incubate at 37C.

The sampling procedure is as follows. Immediately after arrival to our laboratory the wild males are used for electrophoresis; each wild female is placed in a separate culture. One F_1 progeny from each female culture is assayed for each enzyme. Thus, we are sampling two wild genomes from the natural population for each wild fly trapped, except for sex-linked loci which exist in males in single copies.

The main results of our electrophoretic studies are shown in Table 4. The alleles at a given locus are symbolized by numbers which refer to the relative electropho-

retic mobility of the enzymes coded by them. One allozyme, usually the most common, is called 100; the difference in anodal migration between that allozyme and some other one, expressed in millimeters, is added to or subtracted from 100 to represent the second allozyme. (In previous studies of allozyme variation in the *D. willistoni* group we have represented the allozymes with fractional numbers: 0.96, 1.00, 1.03, etc.; we drop now the decimal point for simplicity.)

For each locality at each locus we give in Table 4 the sample size, the allelic frequencies, and the proportion of heterozygous individuals. Some alleles which occur in *D. equinoxialis* in low frequencies are omitted from the Table. The sample size is the number of wild genomes sampled. In the Tortuguero and Mayagüez localities, where more than one sample was available, we have pooled the data from various samples to estimate the allelic frequencies. There is no evidence of significant difference between the samples of a given locality; thus pooling the data from various samples permits a more accurate estimation of the true allelic frequencies. The frequency of heterozygous individuals at each locus (*Heterozygotes*) are their expected frequencies on the assumption of Hardy-Weinberg equilibrium. Generally there are not significant differences between the observed and the expected frequencies of heterozygotes with one notable exception; at the *Est-3* locus in Tortuguero, the average frequency of heterozygotes in the four samples is 0.263, while their expected frequency calculated from the pooled data is 0.339.

We have found allelic variation at every locus studied in *D. e. caribbensis*, although not in every population. The average number of genomes sampled per locus for the subspecies is 1425; even very rare alleles are likely to be occasionally found in our samples. The amount of polymorphism varies, however, from locus to locus over a wide range. At one extreme, the average frequency of heterozygous individuals is two per thousand and six per thousand at the *To* and *αGpdh* loci, respectively. At the other extreme, more than fifty percent of the individuals are heterozygous, on the average, at the *Xdh* and *Acph-1* loci. A similarly wide range in the amount of polymorphism per locus has been found in other taxa of the *D. willistoni* group (Ayala et al., 1972*a*,*b*; Richmond, 1972), and indeed in most other organisms where allozyme variation has been studied at a large number of loci.

The wide range of variation from locus to locus in the amount of polymorphism within a given population, contrasts with the relatively small amount of variation from population to population within a given locus. This situation is again similar to that observed in other species of Drosophila (Prakash et al., 1969; Ayala et al., 1972*a*,*b*).

Table 5 gives a summary of allozyme variation in *D. e. caribbensis*. The proportion of polymorphic loci per population is calculated using two different criteria, one more restrictive than the other. The proportion of heterozygous loci per individual is simply the mean over all loci of the proportion of heterozygous individuals per locus. This latter statistic provides the most precise measure of genetic variation in populations of outcrossing, sexually reproducing organisms. For purposes of comparison between the subspecies, Tables 4 and 5 give summaries of genetic variation in *D. e. equinoxialis*. These data are taken from Ayala et al. (1972*a*).

In *D. e. caribbensis* the mean proportion of heterozygous loci per individual is for the six populations $17.9 \pm 1.10\%$; in *D. e. equinoxialis*, the mean is $21.8 \pm 3.0\%$, or about 4% greater. It can be seen in Table 5, however, that the *D. e. caribbensis* population from Central America, Tortuguero, has about the same amount of genetic polymorphism ($22.2 \pm 3.6\%$ heterozygous loci per individual) as the mean for the 27 continental populations (including Trinidad, a continental island) of *D. e. equinoxialis*; while the island popu-

TABLE 5. *Summary of genetic variation in natural populations of two subspecies of* Drosophila equinoxialis.

	D. e. caribbensis						
	Tortuguero	Santiago	Santo Domingo	Mayagüez	Barranquitas	Yunque	*D. e. equinoxialis*[1]
Number of loci studied	23	26	26	27	26	24	27
Number of genes sampled per locus	332 ± 45	142 ± 8	164 ± 9	332 ± 28	438 ± 31	17 ± 1	1039 ± 177
Polymorphic loci per population (1)[2]	.609	.385	.423	.481	.385	.583	.711 ± .071
(2)[2]	.826	.654	.692	.741	.538	.583	.876 ± .039
Heterozygous loci per individual	.222 ± .036	.158 ± .039	.175 ± .039	.188 ± .039	.146 ± .036	.187 ± .045	.218 ± .030

[1] Including two loci, *Acph-2* and *Odh-2*, not shown in Table 4.
[2] A locus is considered polymorphic (1) when the frequency of the most common allele is $\leqslant 0.95$; (2) when the frequency of the second most common allele is $\geqslant 0.01$.

lations of *D. e. caribbensis* have lower levels of heterozygosity. To compare the overall amount of heterozygosity between populations, we have obtained the difference in heterozygosity at each locus, and then have calculated the mean difference over all loci studied in the pair being compared. The mean differences in heterozygosity are given in Table 6. The Yunque population is not included in these comparisons, because the small size of the samples make the estimates of heterozygosity per locus unreliable.

The island populations of *D. e. caribbensis* are, then, less polymorphic than either the Central American population of *D. e. caribbensis* (average difference in heterozygosity 0.075 ± .007) or the continental populations of *D. e. equinoxialis* (average difference in heterozygosity 0.077 ± 0.004), while there is no significant difference between the latter two in the amount of heterozygosity. Ayala et al. (1971) compared the amount of heterozygosity in four continental and six island populations of *D. willistoni*. The average heterozygosity per individual was somewhat lower in the island (16.2 ± 0.6%) than in the continental populations (18.4 ± 0.9%).

If we look at the kind, rather than at the amount of variation; that is at what alleles are present and in what frequencies in the various populations, we can extend to *D. e. caribbensis* two generalizations which were formulated for other taxa of the *D. willistoni* group (Ayala, 1972; Ayala et al., 1972*a*,*b*). (1) *At any given locus the pattern of the variation remains fairly constant from population to popu-*

TABLE 6. *The mean differences in heterozygosity between the populations.*[1]

	Tortuguero		*D. e. equinoxialis*	
Tortuguero	—		−0.015 ± 0.034	($t = -0.45$, $P > 0.50$)
Santiago	0.081 ± 0.033	($t = 2.47$, $P < 0.05$)	0.068 ± 0.039	($t = 1.73$, $P > 0.10$)
Santo Domingo	0.067 ± 0.032	($t = 2.12$, $P < 0.05$)	0.083 ± 0.039	($t = 2.12$, $P > 0.05$)
Mayagüez	0.059 ± 0.030	($t = 1.97$, $P < 0.10$)	0.073 ± 0.037	($t = 1.96$, $P > 0.10$)
Barranquitas	0.091 ± 0.031	($t = 2.94$, $P < 0.01$)	0.083 ± 0.039	($t = 2.15$, $P > 0.05$)

[1] *Tortuguero* or *D. e. equinoxialis* minus population named in the first column.

lation; one and the same allele is, with few exceptions, the most common throughout the whole distribution of the species. (2) Nevertheless, *the allelic frequencies are not uniform everywhere*; significant differences in the frequencies of some alleles occur between populations.

We have studied one population in Central America and several on two oceanic islands, Hispaniola and Puerto Rico. Yet the pattern of the variation at any given locus is remarkably similar from population to population. The most notable exception to this generalization occurs at the *Ao-1* locus, where the most common allele is *100* in Santo Domingo (frequency 0.840), but *105* elsewhere in the subspecies (frequency 0.904 to 0.919). Another exception is the *Pgm-1* locus; allele *104* is the most common in the island populations (frequency 0.895 to 0.962), but only the second most common (frequency 0.333) in Costa Rica. At each of two other loci, *Est-5* and *Xdh*, the two most common alleles occur in approximately equal frequencies on the average; which one of the two is the most common varies from locality to locality.

Inspection of Table 4 makes it clear that in many instances there are significant differences between localities in the frequencies of a given allele. At the *Ao-1* locus, mentioned above, the frequency of allele *105* ranges from 0.904 to 0.919 in Santiago, Mayagüez and Barranquitas, but has a frequency of 0.038 in Santo Domingo; while allele *100* which has a frequency of 0.840 in Santo Domingo is absent in the other populations. At the *Pgm-1* locus, the frequency of allele *104* is 0.333 in Costa Rica, but 0.895 or greater in the island populations; while allele *100* has a frequency of 0.667 in Costa Rica, but 0.105 or lower elsewhere. Statistically significant differences in the frequencies of one or more alleles occur also at many other loci; for instance, *Lap-5*, *Est-2*, *Est-3*, *Est-4*, *Est-6*, *Acph-1*, *Ald* and *Adk-1*.

As we have argued earlier (Ayala, 1972; Ayala et al., 1971, 1972*a*,*b*) the pattern of the allelic variation indicates that the polymorphisms are maintained by natural selection. The overall similarity of the allelic frequencies in geographically isolated populations is incompatible with the hypothesis that the allozyme variation is adaptively neutral and therefore the allelic frequencies are determined by random processes. The fact that differences between any two localities occur at some but not at other loci excludes the possibility that the overall similarity in the pattern of the variation is due to migration between the populations.

Genetic Differentiation Between the Subspecies

To quantify the amount of genetic differentiation between two populations, we use the following statistics (Nei, 1972). Let X and Y be two different populations, and K a given locus. The normalized probability that two alleles, one from each population, are identical is

$$J_K = \Sigma\, x_i y_i / (\Sigma\, x_i^2\, \Sigma\, y_i^2)^{1/2} \qquad (3)$$

where x_i and y_i are the frequencies of the ith allele in populations X and Y, respectively. The mean *genetic similarity* over several loci is

$$J = J_{xy} / (J_x J_y)^{1/2} \qquad (4)$$

where J_{xy}, J_x and J_y are the arithmetic means, over all loci of $\Sigma\, x_i y_i$, $\Sigma\, x_i^2$, and $\Sigma\, y_i^2$, respectively.

The average genetic differentiation, or *genetic distance* per locus, between the two populations is estimated by

$$D = -\log_e J \qquad (5)$$

Under certain assumptions, the statistic D can be interpreted as the average number of electrophoretically detectable amino acid substitutions *per locus* which have accumulated since populations X and Y had a common ancestral population.

Table 7 gives the genetic distance, D, and the genetic similarity, J, between each pair of populations of *D. e. caribbensis*,

TABLE 7. *Genetic distance (above diagonal) and genetic similarity (below diagonal) between populations of* Drosophila equinoxialis.

		D. e. caribbensis					
	Tortuguero	Santiago	Santo Domingo	Mayagüez	Barranquitas	Yunque	*D. e. equinoxialis*
D. e. caribbensis							
Tortuguero	–	.042	.040	.036	.043	.032	.225
Santiago	.959	–	.038	.003	.003	.017	.266
Santo Domingo	.960	.963	–	.042	.038	.018	.274
Mayagüez	.965	.997	.959	–	.004	.012	.259
Barranquitas	.958	.997	.963	.996	–	.020	.268
Yunque	.969	.984	.982	.988	.981	–	.236
D. e. equinoxialis	.798	.766	.760	.772	.765	.790	–

and between each population of *D. e. caribbensis* and *D. e. equinoxialis*. Very little genetic differentiation has taken place between local populations of *D. e. caribbensis*, even between those in different islands, or between the mainland and the island populations. Greater differentiation exists, on the average, between the Central American population and the island populations ($\bar{D} = 0.039 \pm 0.002$) than between the island populations ($\bar{D} = 0.019 \pm 0.005$). Among the island populations, Santo Domingo is more different from the other populations ($\bar{D} = 0.034 \pm 0.005$) than these are from each other ($\bar{D} = 0.009 \pm 0.003$). This latter difference is largely due to the *Ao-1* locus, where the most common allele in Santo Domingo is *100* (frequency 0.840), an allele which is absent from the other island populations.

The amount of genetic differentiation between populations of two different subspecies is far greater than the differentiation between populations of the same subspecies. The mean genetic distance between the subspecies is $\bar{D} = 0.255 \pm 0.008$, while the mean distance between local populations of *D. e. caribbensis* is $\bar{D} = 0.026 \pm 0.004$, and between local populations of *D. e. equinoxialis* (calculated from data in Ayala et al., 1972*a*) is $\bar{D} = 0.018 \pm 0.002$.

Inspection of Table 4 shows, however, that at most loci the same alleles are found in approximately the same frequencies in the two subspecies. The degree of genetic differentiation between the two subspecies is not due to a moderate amount of genetic differentiation at each of many loci, but rather to complete, or nearly so, differentiation at a few loci while a majority of loci remain essentially identical. We have calculated the average genetic similarity between each population of *D. e. caribbensis* and *D. e. equinoxialis* at each of the 25 loci studied in both subspecies. The distribution of the number of loci with a given degree of genetic similarity is depicted in Figure 1. Only 3 out of 25 loci (12%) have genetic similarities between 0.15 and 0.85, which represents 75% of the total range. At 18 loci (72%) the two subspecies are essentially identical, while at 4 loci (16%) their differentiation is nearly complete.

The four loci at which the differentiation between the subspecies is nearly complete are *Acph-1*, *Mdh-2*, *G3pdh*, and *Hk-1*. Using the method of Ayala and Powell (1972) we have calculated the probability of correct identification of the subspecies of an individual of known genotype. Briefly, this method is as follows. The genotypic frequencies at each locus are calculated from the allelic frequencies in Table 4 assuming random mating equilibrium. Genotypes present only in one subspecies are assigned to that subspecies.

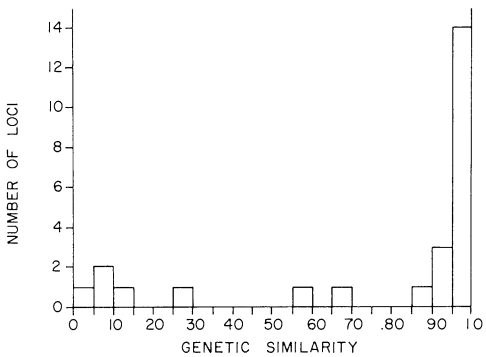

FIG. 1. Histogram showing the number of loci within a given range of values of genetic similarity, when populations of *D. e. equinoxialis* and *D. e. caribbensis* are compared at 25 loci coding for enzymes.

When the same genotype exists in two subspecies, an individual with that genotype is assigned to the subspecies in which that genotype has the higher frequency. If in a given sample, individuals of the two subspecies are equally common, the probability of misclassification of an individual of a given genotype is half the frequency of that genotype in the subspecies in which the genotype is rarer. The addition of this probability over all genotypes at a given locus gives the probability of misclassification of an individual of known genotype using that locus alone for diagnosis. That probability is 0.0003 for *Mdh-2*, 0.014 for *Acph-1*, 0.023 for *G3pdh*, and 0.025 for *Hk-1*. If these four loci are jointly used the probability of incorrect identification of an individual of known genotype is 2.4×10^{-9}. Ayala and Powell (1972) have shown that allozymes can be used as diagnostic characters between sibling species of *Drosophila*. We now extend that finding to subspecies. Genetic differentiation at those four loci (and a fifth one, *Est-4*, giving a probability of misclassification of 0.033) has been used in the formal description of the subspecies *D. e. caribbensis* (Ayala, 1973). This is probably the first time that allozymes have been used as diagnostic characters in the formal description of a taxon.

DISCUSSION

The "mating chamber" experiments show that a small, but statistically significant degree of sexual isolation exists between geographic strains of *D. equinoxialis*. Incipient sexual isolation between allopatric populations of the same species is not a rare phenomenon in *Drosophila*. Anderson and Ehrman (1969) have reviewed the literature, and shown that statistical deviations from random mating between geographically separated populations occur in 19 out of 21 species for which the relevant information exists. The isolation coefficients are significantly greater than zero in 123 (47.9%) out of 257 pairs of strains tested.

Species differ from each in the amount of sexual isolation between their geographic populations. Anderson and Ehrman (1969) used "mating chambers" to study sexual isolation between strains derived from five widely scattered geographic populations of *D. pseudoobscura*; there was no evidence of sexual isolation between any pair of populations. Ayala (1965) found little or no evidence of sexual isolation in 132 pairwise comparisons between 15 geographic strains of *D. serrata*. In *D. birchii*, a sibling species of *D. serrata*, Ayala (1965) found on the contrary that sexual isolation existed between any pair of six strains studied; the coefficient of sexual isolation was statistically greater than zero at the one per thousand level in 12 out of 15 pairwise comparisons, and at the one percent in one more.

The origin of reproductive isolating mechanisms has been reviewed by Mayr (1963) and Dobzhansky (1970). The problem of the origin of sexual isolation between allopatric populations of the same species remains controversial. If the incipient sexual isolation observed in the laboratory is genetically determined, rather than a laboratory artifact, it must arise as a by-product of genetic differentiation between populations adapted to different environments. There seems to be no

way in which natural selection would directly promote the development of sexual isolation between allopatric populations. However, if sexual isolation arises as a by-product of genetic differentiation, the degree of sexual isolation between any two populations might be expected to be directly proportional to the amount of genetic differentiation between them. Yet that is not the case. Populations of different subspecies of *D. equinoxialis* are 10 to 20 times more differentiated genetically than populations of the same subspecies, but the degree of sexual isolation is no greater between the former than between the latter. The existence of incipient sexual isolation between geographic populations of *D. equinoxialis* seems to reflect that the gene pools are different but not *how different* the gene pools are. It may be that the sexual isolation observed in the laboratory between geographic strains simply reflects some ability of the flies to "recognize" members of the same population from individuals of a different population. Another possibility is that the incipient sexual isolation is not due to genetic differences, but rather that flies developing in the same culture "recognize" each other because they have the same "smell."

One of the most important questions in evolutionary genetics is what proportions of gene loci are altered in the evolutionary processes, and particularly in the process of speciation. The techniques of gel electrophoresis allow study of allelic variation at many randomly chosen loci in a population without prohibitive requirements of time and cost. Comparison between populations of a desired degree of evolutionary divergence can thus be made. Since enzyme loci can be chosen for electrophoretic loci independently of whether differences exist or not at the chosen loci in the populations being compared, it can be assumed that such loci represent a random sample of the genome. The degree of genetic differentiation found at a moderate number of loci can then be assumed to be true of the complete genome, at least as a first approximation.

Many closely related species have recently been compared using techniques of gel electrophoresis. We have studied about 30 enzyme loci in the four sibling species, *D. willistoni*, *D. equinoxialis*, *D. tropicalis* and *D. paulistorum*. The average genetic similarity between populations of different species is $\bar{I} = 0.524$; the average genetic distance is $\bar{D} = 0.646$. When populations of any one of the four sibling species are compared with populations of *D. nebulosa*, an easily morphologically distinguishable species of the same group, the average values obtained are $\bar{I} = 0.279$, $\bar{D} = 1.28$. Comparisons of more or less closely related species in other groups of *Drosophila* give estimates of genetic similarity which are of about the same magnitude as those observed in the *D. willistoni* group (Hubby and Throckmorton, 1968; Lakovaara et al., 1972).

Closely related species have also been compared in vertebrates. Although different statistics are used in different reports, the degree of genetic differentiation appears to be of the same magnitude as in *Drosophila* in most organisms adequately studied, including rodents like *Dipodomys* (Johnson and Selander, 1971), and *Peromyscus* (Avise et al., 1974); sunfishes (Avise and Smith, 1974); *Anolis* lizards (Webster et al., 1973); and *Taricha* salamanders (Hedgecock and Ayala, 1974).

The vertebrate as well as the *Drosophila* studies show that a substantial amount of genetic differentiation exists between different species, even when they are closely related. These studies, however, do not answer the question, how much genetic differentiation accompanies the process of species formation. Assume that a certain amount of genetic differentiation exists between two species; there is no way of telling how much of that differentiation occurred before speciation was completed and how much is the result of subsequent divergence. Species are independent evo-

lutionary units. Genetic differentiation is likely to continue after speciation has occurred. The average genetic distance between *D. nebulosa* and the sibling species of *D. willistoni* ($\bar{D} = 1.28$) is almost twice as large as the average genetic distance between sibling species ($\bar{D} = 0.646$, see above).

The generally accepted model of geographic speciation recognizes first a stage at which isolated allopatric populations become genetically different. If such genetically differentiated populations become again sympatric, at least in part, there may be two possible outcomes: (1) ultimately, a single gene pool is formed, either because the two populations readily hybridize, or because one of the gene pools is completely eliminated by competition; (2) two species ultimately arise, because natural selection gradually leads to the development of complete reproductive isolation between the populations owing to the lowered fitness of hybridizing individuals. Which one of these two alternatives will be realized depends of course on the degree of genetic differentiation achieved previous to sympatry.

The two subspecies of *D. equinoxialis* represent instances of substantial genetic differentiation between allopatric populations. Some reproductive isolation exists between the subspecies in the form of postmating isolating mechanisms, that is, sterility of male hybrids. If the two subspecies were to become sympatric, natural selection might favor the development of premating isolating mechanisms between the subspecies. Alleles promoting matings between individuals of the same subspecies would have a fitness advantage over alleles promoting intersubspecific matings, since half of the progeny of the latter matings are sterile. Whether selection would lead to the formation of two different species, or whether a hybrid swarm would be formed cannot, of course, be predicted. Be that as it may, it is clear that the two subspecies represent allopatric genetic differentiation of the kind that if it proceeds far enough may lead to the development of complete reproductive isolation, and thus speciation upon sympatry.

What proportion of the genes have become altered in the process of formation of the subspecies? The mean genetic distance between populations of different subspecies is $\bar{D} = 0.255 \pm 0.008$. That is, about 25.5 electrophoretically detectable allelic substitutions have taken place for every 100 loci. A substantial fraction of the genome has differentiated beween the subspecies. In contrast, the average genetic distance between populations of the same subspecies is $\bar{D} = 0.026 \pm 0.004$ in *D. e. caribbensis*, and $\bar{D} = 0.018 \pm 0.002$ in *D. e. equinoxialis*.

How much genetic differentiation has occurred in the formation of other subspecies? Two subspecies exist in *D. willistoni* (Ayala, 1973), *D. w. quechua* (in Peru, west of the Andes) and *D. w. willistoni*. Some reproductive isolation exists between them, in the form of postmating isolating mechanisms, but it has not advanced as far as between the subspecies of *D. equinoxialis*. Crosses between *D. w. willistoni* females and *D. w. quechua* males yield fertile males and females. Crosses with *D. w. quechua* as the female parent produce fertile female hybrids, but sterile hybrid males when the male parent comes from Colombia, Venezuela, Trinidad or Brazil. We have studied allozyme variation at 25 loci in both subspecies. The genetic distance between populations of the two subspecies is $\bar{D} = 0.202$. In contrast, the average genetic distance between populations of the same subspecies is $\bar{D} = 0.015$ in *D. willistoni*.

Incipient reproductive isolation between certain allopatric populations exists also in *D. pseudoobscura* (Prakash, 1972). Males from Bogotá crossed to females from any other locality yield fertile males and females; the reciprocal crosses produce fertile females but sterile F_1 males. Genetic variation has been studied at 24 protein loci in *D. pseudoobscura* by Prakash, Lewontin, and Hubby (1969). Using these

authors' data (see also Nei, *in press*) we have estimated the mean genetic distance between Bogotá and populations from the main area of the distribution of the species as $\bar{D} = 0.110 \pm 0.014$, while the mean genetic distance between populations other than Bogotá is $\bar{D} = 0.007 \pm 0.002$. Thus about 15 times more genetic differentiation exists between the Bogotá population and the rest of the species, than between geographic populations of *D. pseudoobscura* which are not reproductively isolated at all. Prakash (1972) has indicated that no alleles have been found in Bogotá which do not also exist elsewhere in *D. pseudoobscura*. Presence or absence of alleles in two populations does not give indication of how genetically different they are, unless the frequencies of the alleles and the sample size are taken into consideration. The data of Prakash et al. (1969) show that, for instance, at the *Pt-8* locus there is little overlap between the *genotypic* frequencies of the Bogotá and the other populations. If the genotype of an individual at the *Pt-8* locus is known, the probability of correct identification of the individual as coming from the Bogotá or from some other population can be calculated from the data of Prakash et al. (1969) as greater than 98%.

To summarize, it seems that, in *Drosophila*, allopatric populations with some mutual reproductive isolation of the kind that might give origin, upon sympatry, to the development of complete reproductive isolation, have average genetic distances, $\bar{D}$, which range from 0.110 to 0.255. Between 11.0 and 25.5 electrophoretically detectable allelic substitutions have occurred for every 100 loci. In the same species the average genetic distance between consubspecific populations ranges from 0.007 to 0.026, or 10 to 15 times smaller.

Genetic variation at 41 protein loci has been studied in two subspecies of the house mouse, *Mus musculus musculus* and *M. m. domesticus* (Selander et al., 1969). We have calculated (see also Nei, 1972) the average genetic distance between populations of different subspecies as $\bar{D} = 0.171 \pm 0.009$. Hedgecock and Ayala (1974) have studied genetic variation at 18 enzyme loci in two subspecies of salamanders, *Taricha torosa torosa* and *T. t. sierrae*. The average genetic distance between populations of different subspecies is $\bar{D} = 0.168 \pm 0.042$. Based on the limited number of the studies available, it appears then that the amount of genetic differentiation between subspecies of vertebrates is of approximately the same magnitude as between subspecies of *Drosophila*, that is, between 10 and 25 electrophoretically detectable allelic substitutions for every 100 loci. The actual degree of genetic differentiation between subspecies is probably greater than those values, since only a fraction of all allelic substitutions are detectable by electrophoresis.

Addendum

D. Poulson and F. Tortora (Poulson and Oishi 1973, pers. comm.) have recently discovered differences between the subspecies *D. e. equinoxialis* and *D. e. caribbensis* in the properties of androcidal infections carried by so-called "sex-ratio" (SR) females. Some wild flies of the *D. willistoni* group produce only female progenies. This hereditary phenomenon is characterized by the presence in the hemolymph of SR females of spiral microorganisms referred to as "SR-spirochetes." When the hemolymph of SR females belonging to different species is mixed in the laboratory, the SR spirochetes "clump" into dense associations of many individual particles. The clumping generally does not occur when the hemolymph of SR females of different strains of the same species is mixed. Clumping has now been found to occur when the hemolymph of SR females of *D. e. equinoxialis* is mixed with that of SR females of *D. e. caribbensis*. The clumping of spirochetes does not, however, occur when the hemolymphs of SR females of different strains of the same *D. equinoxialis* subspecies are mixed. There is also indication that the frequency of SR females in natural populations is greater in *D. e. caribbensis* than in *D. e. equinoxialis*.

Summary

Drosophila equinoxialis consists of two subspecies, *D. e. equinoxialis* and *D. e. caribbensis*, that are partially reproduc-

tively isolated from each other. Crosses between the subspecies yield fertile females but sterile males.

We have studied allelic variation at 27 loci coding for enzymes in six natural populations of *D. e. caribbensis*. Like other taxa of the *D. willistoni* group, *D. e. caribbensis* is very polymorphic. On the average, the proportion of heterozygous loci per individual is $17.9 \pm 1.1\%$; the proportion of polymorphic loci per population is $47.8 \pm 4.0\%$, or $67.2 \pm 4.3\%$ depending on whether a more or less restrictive criterion of polymorphism is used.

Populations of *D. e. caribbensis* from Hispaniola and Puerto Rico are somewhat less polymorphic than a population from Central America, but the latter is as polymorphic as continental populations of *D. e. equinoxialis*.

The average genetic distance between any two local populations of *D. e. caribbensis* is 0.026 ± 0.004, and between any two local populations of *D. e. equinoxialis* is 0.018 ± 0.002. In contrast the average genetic distance between the subspecies is much greater, 0.255 ± 0.008. In other *Drosophila* species, as well as in the house mouse and in *Taricha* salamanders, the genetic distance between subspecies ranges from 0.110 to 0.202, while the difference between local populations of the same subspecies is about 10–15 times smaller. Subspecies are allopatric populations partially reproductively isolated from each other. They represent an intermediate stage in the process of geographic speciation. Between 11.0 and 25.5 electrophoretically detectable allelic substitutions for every 100 loci occur in the process of differentiation of two subspecies.

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