INVERSION CLINES IN POPULATIONS OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Twenty different natural populations of *Drosophila melanogaster* were sampled to determine the frequencies of inversions. Based on their frequencies and geographical distributions, the inversions could be classified as follows: (1) *Common cosmopolitan* inversions that are present in many populations in frequencies exceeding five percent and that may exhibit frequency clines over large geographical regions; (2) *Rare cosmopolitan* inversions that occur throughout the species range but usually at frequencies below five percent and that may be absent in many populations; (3) *Recurrent endemic* inversions that are found in several adjacent populations in frequencies usually not exceeding one or two percent; and (4) *Unique endemic* inversions that are found only among the progeny of a single individual and that may represent one aspect of the syndrome termed "hybrid dysgenesis". Four common cosmopolitan inversions that exhibit highly significant clines in populations in the eastern United States are *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P*.

NOTWITHSTANDING the extensive use of *Drosophila melanogaster* as an experimental organism in population genetics, relatively little is known about the patterns of inversion polymorphisms that exist in this species. While a number of studies (DUBININ, SOKOLOV and TINIAKOV 1937; WARTERS 1944; IVES 1947; MOURAD and MALLAH 1960; OSHIMA, WATANABE and WATANABE 1964; WATANABE 1967; YANG and KOJIMA 1972; STALKER 1976; ASHBURNER and LEMEUNIER 1976) have shown that inversions are not rare and may even reach high frequencies in some local populations, little is known about inversion frequency patterns over large geographical regions. Knowledge of inversion clines may be important in answering the general question of what factors are involved in the maintenance of allozyme variation in Drosophila, since it is known that linkage disequilibria between inversions and allozyme loci are not uncommon in this species (KOJIMA, GILLESPIE and TOBARI 1970; MUKAI, METTLER and CHIGUSA 1970, 1971; MUKAI, WATANABE and YAMAGUCHI 1974; MUKAI and VOELKER 1977; LANGLEY, TOBARI and KOJIMA 1974; LANGLEY, ITO

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and VOELKER 1977; VOELKER, MUKAI and JOHNSON 1977; SCHAFFER *et al.*, in preparation).

In the present paper we present evidence that natural populations of *D. melanogaster*, primarily along the eastern seaboard of the United States, exhibit north–south clinal patterns for some polymorphic inversions, with southern populations having higher inversion frequencies than northern populations.

MATERIALS AND METHODS

The flies used in this study were collected by sweeping an insect net over fallen rotting fruit which was in season at the time or by traps that contained rotting fruit. The sources of the flies and the dates of collection are listed in Table 1.

When the wild-collected flies were to be used to provide data only for this study, single males were crossed to several virgin females homozygous for cytologically standard sequence in all chromosomes. Slides of the salivary gland chromosomes of one or more larvae from these crosses were analyzed. The lactic-acetic-orcein staining procedure was used. When more than one F_1 larva per male was analyzed, the sample size was adjusted to indicate the probable number of chromosomes sampled. (The estimated number of chromosomes sampled per male when more than one F_1 larva was used was $2-(\frac{1}{2})^{n-1}$, where n equals the number of F_1 larvae examined.) If the number of wild-caught males was insufficient, single F_1 males of wild-inseminated females were sometimes used to augment the sample size. When such males were used, they were assumed to provide equivalent information to wild-caught males.

Sometimes the wild-collected males (or F_1 males of wild-inseminated females) were used to extract second and/or third chromosomes for studies of allozyme variation and/or linkage

TABLE 1

Collection sites and dates

City and State (Country)	Date(s)
San Juan, Puerto Rico	January, 1972
Mexico City, Mexico	May, 1972
Miami, Fla.	March, June, 1971
Lake Placid, Fla.	December, 1971
Orlando, Fla.	December, 1971
Lake Wales, Fla.	May, 1974
Jacksonville, Fla.	September, November, 1971
Tucson, Ariz.	June, 1972
Mesa, Ariz.	June, 1971
Lubbock, Texas	September, 1970
Columbia, S. C.	August, 1971
Angier, N. C.	October, 1971
Raleigh, N. C.	September, 1968; June, September, 1969; July, 1970
Knoxville, Tenn.	July, 1970
Wachapreague, Va.	July, 1971
Winchester, Va.	September, 1971
Crete, Nebr.	July, 1972
Lincoln, Nebr.	September, 1972
Niagara Falls, N. Y.	September, 1971
Portland, Maine	August, 1971

disequilibrium. In such cases the salivary gland chromosomes were analyzed after the lines had been established and were being maintained over balancer second and/or third chromosomes.

RESULTS AND DISCUSSIONS

Types and frequencies of inversions: The inversion frequencies found in the various localities sampled are given in Table 2. The designations of the inversions follow the nomenclature of LINDSLEY and GRELL (1968), except that *In(2R)NC* is a newly described inversion first found in Raleigh, North Carolina, populations. The column headed "Uniques" will subsequently be explained and discussed. The collection sites are listed in order of increasing northern latitude location.

Considering their geographic ranges and frequencies from this work, as well as from previous studies, inversions may be categorized as follows:

I. *Cosmopolitan*: These are inversions which have been observed in populations from all parts of the species' geographical range. Cosmopolitan inversions include those that are at present polymorphic in many populations throughout the species range or may be remnants of historically prevalent polymorphisms (thereby achieving their distributions throughout the species range). Genetically, they probably contribute to the segregational genetic load. The two types of cosmopolitan inversions have been denoted as *common* or *rare*.

A. *Common cosmopolitan* inversions are often found in frequencies greater than five percent, on rare occasions being more frequent than the Standard sequence. *In(2L)t*, *In(2R)NS*, *In(3L)P*, and *In(3R)P* are included in this category. Considering the various populations of this study, it is evident that these four inversions occur with higher frequencies in southern populations and are found less frequently or are absent in northern populations (see below). *In(3R)P* is unusual in that in two large samples from central Florida it is much more frequent than the Standard sequence; in all other cases the Standard sequence is more frequent than the respective common cosmopolitan inversion sequence.

B. *Rare cosmopolitan* inversions are nearly always found in frequencies below five percent and may be absent in many localities. Included in this grouping are *In(2R)Cy*, *In(3L)M*, *In(3R)C*, *In(3R)K*, *In(3R)Mo*, *In(3R)M* and *In(2L)NS* (the latter was not observed in this study). Also included in this grouping is *In(2L)Cy*; this inversion was not specifically observed in this study, but because of its great similarity to *In(2L)t* it is possible that a rare *In(2L)Cy* might have been misidentified as *In(2L)t*. All 46 putative *In(2L)t* inversions in MUKAI and VOELKER (1977) and VOELKER, MUKAI and JOHNSON (1977) were verified to be *In(2L)t*. [See also the comments of ASHBURNER and LEMEUNIER (1976) on *In(2L)Cy*.] Possibly also included in this grouping is *In(2L)22A;26A* of ASHBURNER and LEMEUNIER (1976), although this is not yet well documented.

II. *Endemic*: These are inversions that are geographically restricted in occurrence and are nearly always found in frequencies not exceeding one or two percent. They are probably relatively newly arisen inversions that, if advan-

TABLE 2

Inversion frequencies in North American populations of Drosophila melanogaster

Location	N§	*In(2L)t*	*In(2R)NS*	*In(2R)NC*	*In(3L)P*	*In(3L)M*	*In(3R)P*	*In(3R)C*	*In(3R)K*	*In(3R)Mo*	*In(3R)M*	Uniques
San Juan, P.R.	41 (60)	0.048	0.000	0.000	0.012	0.000	0.434	0.000	0.024	0.000	0.000	0.000
Mexico City, Mex.	30	0.000	0.200	0.000	0.033	0.000	0.067	0.033	0.000	0.067	0.000	0.067
Miami, Fla.	114	0.246	0.316	0.000	0.149	0.009	0.474	0.009	0.009	0.000	0.000	0.000
Miami, Fla.	336	0.253	0.235	0.000	0.110	0.000	0.417	0.000	0.000	0.000	0.000	0.015
Lake Placid, Fla.	70	0.186	0.214	0.000	—	—	—	—	—	—	—	0.000*
Lake Placid, Fla.	455	—	—	—	0.059	0.000	0.793	0.000	0.000	0.000	0.000	0.020†
Lake Wales, Fla.	745	0.150	0.183	0.000	0.046	0.000	0.804	0.000	0.000	0.000	0.000	0.000[0.105]‖
Orlando, Fla.	395	0.185	0.225	0.000	—	—	—	—	—	—	—	0.025*
Jacksonville, Fla.	42 (80)	0.144	0.264	0.000	0.088	0.012	0.212	0.028	0.000	0.000	0.008	0.032
Jacksonville, Fla.	263	0.099	0.133	0.000	0.046	0.000	0.194	0.019	0.008	0.000	0.019	0.077
Tucson, Ariz.	72	0.278	0.236	0.000	0.194	0.000	0.236	0.042	0.000	0.000	0.000	0.028
Mesa, Ariz.	100	0.100	0.250	0.000	0.090	0.000	0.240	0.030	0.000	0.000	0.000	0.030
Lubbock, Texas	99 (173)	0.199	0.178	0.000	0.199	0.010	0.195	0.027	0.020	0.000	0.011	0.050
Columbia, S.C.	100	0.080	0.080	0.000	0.010	0.000	0.130	0.010	0.000	0.000	0.000	0.060
Angier, N. C.	187	0.054	0.096	0.000	0.016	0.000	0.118	0.011	0.000	0.000	0.000	0.011
Raleigh, N. C.	316	0.076	0.095	0.013	—	—	—	—	—	—	—	0.044*
Raleigh, N. C.	148	0.021	0.095	0.000	—	—	—	—	—	—	—	0.027*
Raleigh, N. C.	276	0.036	0.130	0.004	0.033	0.000	0.112	0.014	0.000	0.000	0.000	0.025
Raleigh, N. C.	152 (259)	0.051	0.086	0.007	0.018	0.000	0.118	0.005	0.000	0.000	0.000	0.044
Knoxville, Tenn.	199 (353)	0.042	0.126	0.010	0.020	0.000	0.112	0.008	0.000	0.000	0.000	0.033
Wachapreague, Va.	57	0.000	0.070	0.018	0.070	0.000	0.140	0.000	0.000	0.000	0.000	0.017
Winchester, Va.	130	0.023	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.008
Crete, Nebr.	22	0.045	0.045	0.000	0.091	0.000	0.091	0.000	0.045	0.000	0.000	0.000
Lincoln, Nebr.‡	150	0.107	0.147	0.000	0.073	0.000	0.153	0.007	0.013	0.007	0.007	0.027
Niagara Falls, N.Y.	215	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.005	0.019
Portland, Me.	127	0.024	0.032	0.000	0.016	0.000	0.032	0.000	0.008	0.047	0.000	0.000

N refers to the number of gametes sampled.

* Frequency is for second chromosome only.

† Frequency is for third chromosome only.

‡ *In(2R)Cy* was found with a frequency of 0.007.

§ The number in parentheses indicates the estimated sample size when more than one larval offspring per male was analyzed.

‖ The number in brackets indicates the frequency of uniques in extracted chromosomes; the number in front of the brackets indicates the unique frequency when wild-caught males were outcrossed.

tageous, have not yet had time to become polymorphic and cosmopolitan. On the other hand, if they are deleterious, they comprise part of the mutational genetic load and probably will be lost. The endemics may be subdivided into categories denoted as *recurrent* and *unique*.

A. *Recurrent endemic* inversions are observed more than once in a given local population or may be observed in adjacent populations within a part of the species range. This category includes *In(2R)NC* (breakpoints are 46A and 49F) from this study, $In(2L)M_1$ and $In(2L)M_2$ of MOURAD and MALLAH (1960), *In(2L)A* of WATANABE (1967) and *In(2R)D*, *In(2R)O* and *In(3L)L* of STALKER (1976). *In(2R)NC* is interesting in that it arose in a chromosome carrying *In(2R)NS* and was always found associated with it. Cage studies (unpublished) suggest that it reached its present frequency and distribution because of its being carried along with *In(2R)NS*.

B. *Unique endemic* inversions are observed only among the progeny of a single individual from a single population. Examples of these have been reported by MOURAD and MALLAH (1960), WATANABE (1967), YANG and KOJIMA (1972), STALKER (1976) and ASHBURNER and LEMEUNIER (1976). It should be noted that translocations, duplications and deficiencies are also rarely recovered as "Unique" aberrations. In this study over 150 such aberrations (nearly all inversions) were observed; they were pooled in the "Unique" column of Table 2.

These "Uniques" are quite probably associated with the syndrome termed "hybrid dysgenesis" by KIDWELL and KIDWELL (1976) and SVED (1976), which is evidenced when chromosomes from some strains (frequently wild strains) are introduced into cytoplasms of other (frequently laboratory) strains. The chromosome aberrations which VOELKER (1974), YAMAGUCHI, CARDELLINO and MUKAI (1976) and YAMAGUCHI (1976) attributed to mutator factors probably represent one facet of "hybrid dysgenesis."

This effect may be amplified if chromosomes from wild flies are extracted (requiring several generations of crosses to strains with cytoplasms of laboratory origin). Note in Table 2 that among the higher frequencies of "Uniques" are those of the Lake Placid, Florida (0.020 for the second chromosome), Orlando, Florida (0.025 for the third chromosome), and Jacksonville, Florida (0.077 for second and third chromosomes); these frequencies were determined in chromosomes which had been extracted. Also noteworthy is the Lake Wales sample where no "Uniques" were observed when wild males ($n = 242$) were outcrossed to females of a laboratory strain, while the frequency of "Uniques" was 0.105 (for second and third chromosomes) when extracted chromosomes ($n = 503$) were analyzed. Thus, some unique aberrations may be artifacts of the crossing scheme used to detect inversions. That this cannot account for all unique endemic inversions, however, is evidenced by the fact that ASHBURNER and LEMEUNIER (1976) also detected such inversions while observing chromosomes from lines that apparently had not been crossed to laboratory strains. There does, however, remain the possibility that their "mass strains" might have contained mixtures of flies which when interbred would give rise to progeny exhibiting "hybrid dysgenesis."

TABLE 3

Common cosmopolitan inversion frequency and location in degrees north latitude of populations sampled in the eastern United States‖

Location	°N. Lat.	*In(2L)t*	*In(2R)NS*	*In(3L)P*	*In(3R)P*
Miami, Fla.	25.87	0.246	0.316	0.149	0.474
Miami, Fla.	25.87	0.253	0.235	0.110	0.417
Lake Placid, Fla.	27.17	0.186†	0.214†	0.059†	0.793†
Lake Wales, Fla.	27.92	0.150†	0.183†	0.046†	0.804†
Orlando, Fla.	28.50	0.185†	0.225†	—	—
Jacksonville, Fla.	30.25	0.144	0.264	0.088	0.212
Jacksonville, Fla.	30.25	0.099	0.133	0.046	0.194
Columbia, S. C.	34.00	0.080	0.080	0.010	0.130
Angier, N. C.	35.50	0.054	0.096	0.016	0.118
Raleigh, N. C.	35.77	0.076‡	0.095‡	—	—
Raleigh, N. C.	35.77	0.021‡	0.096‡	—	—
Raleigh, N. C.	35.77	0.036	0.130	0.033	0.112
Raleigh, N. C.	35.77	0.051	0.086	0.018	0.118
Raleigh, N. C.	35.77	0.057§	0.114§	0.012§	0.088§
Raleigh, N. C.	35.77	0.070*	0.151*	—	—
Knoxville, Tenn.	35.97	0.042	0.126	0.020	0.112
Wachapreague, Va.	37.67	0.000	0.070	0.070	0.140
Winchester, Va.	39.23	0.023	0.000	0.000	0.015
Niagara Falls, N. Y.	43.08	0.000	0.000	0.005	0.000
Portland, Me.	43.67	0.024	0.032	0.016	0.032
Correlation coefficient on °N. Lat.		—0.92***	—0.90***	—0.74***	—0.80***

* Data from Mukai and Voelker (1977).
† Data from Schaffer *et al.*, in preparation.
‡ Data from Mukai, Mettler and Chigusa (1971).
§ Data from Mukai, Watanabe and Yamaguchi (1974).
‖ The bottom row contains the correlation value (r) between the respective inversions and latitude.
*** Significant at $P < 0.001$.

Inversion clines in populations from the eastern United States. Since many populations along or within several hundred miles of the eastern seaboard had been sampled, the data were analyzed for evidence of north-south clines. In Table 3 are presented inversion frequencies of the four common cosmopolitan inversions and the locations in degrees north latitude of the populations sampled. It is evident that *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P* show highly significant north-south clines, having, respectively, r values of —0.92***, —0.90***, —0.74***, and —0.80***. The higher frequencies of common cosmopolitan inversions in southern United States populations are consistent with results reported by Warters (1944), Yang and Kojima (1972) and Stalker (1976). Of the above three studies, only the data of Stalker (1976) are suitable for examination for the presence of a north-south cline; there is no clear evidence of such a cline from Webster Groves, Missouri, to Waverly and Tallulah, Louisiana.

Johnson and Schaffer (1973) have pointed out that both average annual precipitation and average annual temperature are negatively correlated with latitude. Watanabe and Watanabe (1973, 1976) reported that *In(2L)t/*

Standard heterokaryotypes had significantly greater female productivity than the two respective homokaryotypes, and STALKER (1976) found that, in two populations, females heterozygous for inversions in several chromosome arms had greater sperm loads than females heterozygous in only one arm or carrying no inversions. At present it is unknown whether these factors (or others) and/or whether migration and drift may play primary roles in determining inversion frequencies. The selective importance of inversions in other species would suggest that selection plays a major role in determining inversion frequencies in *D. melanogaster.*

The finding that there are polymorphic inversions whose frequencies exhibit clinal patterns of variation may be of significant importance in interpreting what forces maintain the clinal patterns at some allozyme loci (JOHNSON and SCHAFFER 1973; SCHAFFER and JOHNSON 1974). The cytogenetic locations of some allozyme loci within or closely adjacent to certain of these common cosmopolitan inversions suggest that the inversion clines and allozyme clines might be interrelated. The analyses of these relationships will be presented in a subsequent paper.

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