GENETIC COADAPTATION IN THE CHROMOSOMAL POLYMORPHISM OF *DROSOPHILA SUBOBSCURA*. I. SEASONAL CHANGES OF GAMETIC DISEQUILIBRIUM IN A NATURAL POPULATION

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Manuscript received December 7, 1982
Revised copy accepted August 15, 1983

ABSTRACT

Seasonal changes in gene arrangement and allozyme frequencies have been investigated in *Drosophila subobscura* for several years. Some arrangements (O_{st} and O_{3+4+7}) show seasonal variation, which suggests that chromosomal polymorphism is flexible in this species. Seasonal changes in allozyme frequencies for *Lap* and *Pept-1* loci, both located within the same inversions of chromosome *O*, are significant only inside the O_{st} arrangement, but not inside O_{3+4} arrangement. This arrangement-dependent response of allozyme generates variation in arrangement-allozyme disequilibrium. The historical hypothesis on the maintenance of disequilibria cannot explain these seasonal changes, and some kind of natural selection must be invoked. Association between *Lap* and *Pept-1* is also seasonal inside O_{st} but not inside O_{3+4}. We propose that O_{st} probably consists of a finite array of supergenes that are differentially favored in each season by natural selection. The present evidence on this supergene selection and other genetic, biogeographic and phylogenetic data points to O_{3+4} as the most primitive gene order among the present arrangements.

GENES do not act independently. Rather, they tend to organize themselves in functional gene complexes or supergenes (DARLINGTON and MATHER 1949), which confer an advantage to the recipient genotypes. Chromosomal inversions play a major role as promoters of supergenes. The maintenance of chromosomal polymorphisms has been explained traditionally by postulating that each chromosomal arrangement represents a coadapted set of genes that evolved locally to produce heterokaryotypic overdominance under particular environmental conditions (DOBZHANSKY 1949, 1950). This concept of geographical coadaptation has been operative in many cases, but in *D. subobscura* this kind of adaptation has been difficult to detect (MCFARQUHAR and ROBERTSON 1963; PENTZOS-DAPONTE and SPERLICH 1965). WASSERMAN (1972) summarized the three most plausible selective hypotheses to explain the maintenance of chromosomal polymorphism. Briefly, selection can operate on inversions at three different levels: chromosomes, individual genes and super-

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genes. Chromosomal or karyotypic selection postulates that each arrangement is adapted to a particular environment. On the other hand, genic selection explains the selective value of arrangements simply by dominance or overdominance of the genes captured by inversions, without the need of internal interactions in the genic content.

The supergene selection postulates that the order and the epistatic interactions among genes are the crucial points to generate coadaptation inside inversions. This hypothesis is complicated when one considers that there may coexist different gene arrays (supergenes) in each kind of arrangement. In this case, recombination in homokaryotypes would produce low fitness gametes (non-coadapted) and induce heterosis as a by-product. This hypothesis of supergene selection (WASSERMAN 1968) has not been tested adequately, and WASSERMAN (1972), using unfavorable material, did find a small, but highly significant, supergene selection in *D. subobscura*. This emphasizes that the concept of coadaptation is still an open issue and that the mechanisms of selection leading to formation and maintenance of supergenes remain largely unsolved.

LEWONTIN (1974) suggested that persistence of allozyme gametic disequilibrium of the same sign in space and in time must be taken as proof of selection. Geographical studies [see HEDRICK, JAIN and HOLDEN (1978) and BARKER (1979) for a review] show that gametic disequilibrium among allozyme loci is rare except when recombination is low. On the other hand, the reported nonrandom association between inversions and allozymes contained in them seems to prove coadaptation, but some complications exist. Several authors (ZOUROS *et al.* 1974; PREVOSTI 1978; FONTDEVILA 1978; FONTDEVILA, MÉNDEZ and ENRIQUEZ 1979; CHARLESWORTH *et al.* 1979; LOUKAS, KRIMBAS and VERGINI 1979) have reported that two allozyme loci, leucyl amino peptidase and peptidase-1 (*Lap* and *Pept-1*), included in the O_{3+4} inversion zone of *D. subobscura* show significant gametic disequilibrium with chromosome *O* arrangements. This association is of the same kind in all populations studied, and this may be taken as a proof of selection for allozymes. However, this and similar cases of association of allozymes with inversions can also be interpreted by postulating that, at the moment of its occurrence, the new inversion captured an array of allozymes that immediately produced a complete disequilibrium. Those allozymes were isolated from the rest of the gene pool by lack of recombination with it. Only new mutations, double crossovers and gene conversions may introduce new allozymes in the new inversion and start a slow process of decay of the association. Thus, if the inversion appeared relatively recently, we will be witnessing a historical gametic disequilibrium in the absence of coadaptation (ISHII and CHARLESWORTH 1977). Moreover, if we allow for migration of the new inversion, the same gametic disequilibrium will be present everywhere.

To avoid these historical difficulties, we have approached the problem by studying temporal changes in one natural population. The rationale of this approach is based on the generally accepted assumption that genetic drift can not be the cause of repeated seasonal changes in either gene frequencies or gametic disequilibrium. We have observed these repeated seasonal variations

during several years and interpret them in terms of genetic coadaptation. Our results demonstrate that supergene selection is most probably operating in our population, but karyotypic and genic selection cannot be ruled out. This is the first unambiguous proof of coadaptation in the chromosomal polymorphism of *D. subobscura* and also throws new light on the problems of the origin of inversions and of the adaptive role of allozymes included in them.

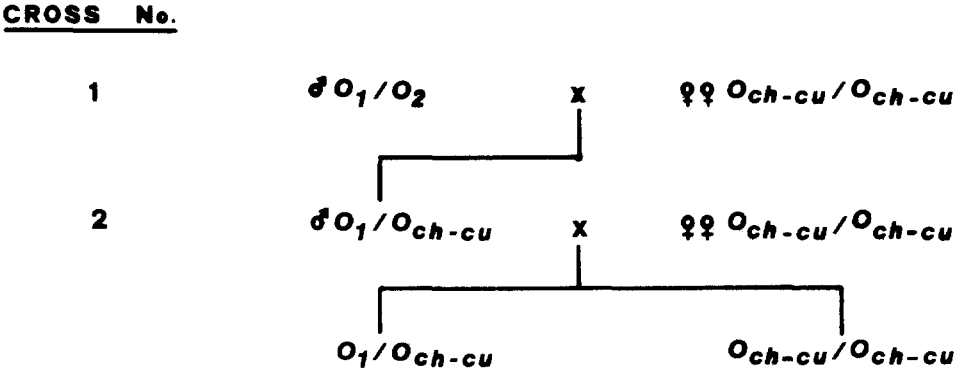
MATERIALS AND METHODS

Population sampling: One natural population of *D. subobscura* located at the south slopes of El Pedroso mountain, 3 km from Santiago de Compostela (Spain), was sampled periodically from the autumn of 1976 until the autumn of 1980. Samples were taken at a pine forest (*Pinus pinaster*), sweeping over conventional banana baits. Dates of sampling were chosen to coincide with four significant seasons in the yearly active period in natural populations of *D. subobscura* at this latitude: spring, early summer, late summer and autumn. The exact collecting dates are as follows:

		Summer		
Year	Spring	Early	Late	Autumn
1976				12/13
1977	4/13		8/30	11/12
1978	3/9	6/14	8/31	11/14
1979	5/8	6/15	8/30	11/29
1980	4/9	6/21	8/28	11/9

Genetic sampling: The genetic polymorphism of the *O* chromosome was computed for all of the arrangements and for three allozyme loci, *Lap*, *Pept-1* and acid phosphatase (*Acph*), included in it (LOUKAS *et al.* 1979). Identification of chromosome arrangements was done by direct observation of polytene chromosomes that had been previously squashed on slides. The preparation technique consisted of dissection of salivary glands of third instar larvae on acetic-alcohol solution (ethanol:60% acetic acid, 3:1). Then, the glands were stained on lactic-orcein (lactic acid:acetic-orcein, 3:1). Identification of allozymes was done by reading of electrophoretic gels that were run and stained according to the techniques of AYALA *et al.* (1972) and LOUKAS *et al.* (1979). Adults were used for *Acph* gels, whereas late pupae were electrophoresed for *Lap* and *Pept-1* detection.

Gametic sampling: To identify gametic types, the following series of crosses were performed:



The series starts by crossing one wild male (O_1/O_2) to females of the *ch-cu* strain. This strain was kindly sent to us by D. SPERLICH. It carries two morphological recessive markers (*ch*, *cu*) in the *O* chromosome (KOSKE and MAYNARD SMITH 1954). This strain is homozygous for the most

frequent electromorph of some allozyme loci (*Lap, Pept-1, Acph, Est, Ao, Xdh, Me*) and is also homokaryotypic for the O_{3+4} arrangement.

Offspring of cross 1 will carry one whole wild *O* chromosome (O_1), since in males recombination is negligible. Males of the F_1 generation are crossed individually to *ch-cu* females, and offspring heterozygotes are electrophoresed. These heterozygotes are phenotypically distinguished from *ch-cu* offspring in adults by their wild-type appearance and in late pupae because mutants develop cherry eyes at this stage. This allows for identification of allozymes in wild *O* chromosomes by comparison with the allozyme content of *ch-cu* chromosomes in the heterozygotes.

The identification of chromosome arrangements in the wild *O* chromosomes must be made by sacrificing the third instar larvae, prior to the time when the allozyme identification is possible. This complication is circumvented by analyzing eight larvae of the F_2 progeny. In this sample the probability of sampling eight homozygous *ch-cu* larvae is less than 0.01. We accept this as our sampling error, and when all eight larvae are homokaryotypic for O_{3+4} we consider that the wild chromosome is carrying this arrangement.

Measures of gametic disequilibrium: Two kinds of gametic disequilibrium can be found: absolute and complete. In a two-locus (*A, B*) two-allele (A_1A_2-B_1B_2) system the absolute association requires that, if A_1 is associated with B_1, the A_2 is associated with B_2. On the other hand, complete association doesn't show this reciprocal relationship and, for example, A_1 can be associated with B_1 without A_2 being associated with B_2 (CLEGG *et al.* 1976; KENDALL and STUART 1961).

Two indices have been reported to quantify gametic disequilibrium, one (D') for complete association and the other (r) for absolute association. Index D' derives from index D, which is defined by the covariance of allelic frequencies. It is computed as the difference between the products of gametic frequencies in coupling and in repulsion. To make D values less dependent of gene frequencies, LEWONTIN (1964) proposed the D' statistic, defined as the ratio D/D_{max}, where D_{max} represents the maximum value of D for each set of gene frequencies.

The correlation coefficient (r) is related to D by the function $r = D/[p_1p_2(1 - p_1)(1 - p_2)]^{0.5}$ where p_1 and p_2 are the allelic frequencies at the two loci. It differs from D in that it is less dependent on gene frequencies and that for identical values of gene frequencies at both loci it ranges from -1.0 to 1.0.

RESULTS

Chromosomal seasonality: During more than 4 yr, frequencies of chromosomal arrangements have been computed at different seasons of the year (Table 1). This represents a total of about 3000 chromosomes checked for inversions with a mean of 186 chromosomes per observation. The two most common arrangements are (in order) O_{3+4+7} and O_{st} with mean frequencies of 0.469 and 0.192, respectively. O_{3+4+8} and O_{3+4} arrangements occur in significant frequencies (>0.10) in general, but the rest of the arrangements of the O_{3+4} phylad are rare and in some cases appear only in a few samples.

Most interesting is the antagonistic seasonal variation found between O_{st} and O_{3+4+7} (Figure 1). Arrangement O_{3+4+7} increases in frequency in summer and decreases in autumn and spring. On the other hand, the O_{st} arrangement shows the opposite change over time. Other arrangements (O_{3+4+8}, O_{3+4}; Figure 1) do not show significant and repeatable seasonal changes.

Allozyme seasonality: Table 2 shows the electromorph frequencies for each sample in detail. The *Acph* and the *Pept-1* frequencies do not show significant changes between seasons. Among the *Lap* electromorphs, only the $Lap^{1.11}$ allele shows a slight seasonal increase in frequency from early summer to autumn (Figure 2b).

Gametic disequilibrium: The analyses of electromorph frequencies have been performed with the view that the allozyme loci are located inside the inverted

TABLE 1

Arrangement frequencies and number of sampled genomes by year and season in the natural population of El Pedroso

O arrangement by segments (I + II)	Year	Season[a]			
		S	ES	LS	A
St (I_{st} + II_{st})	1976				0.237
	1977	0.267		0.138	0.309
	1978	0.191	0.161	0.169	0.237
	1979	0.242	0.150	0.120	0.213
	1980	0.195	0.119	0.137	0.190
7 (I_{st} + II_7)	1976				0.019
	1977	0.023		0.023	0.025
	1978	0.032	0.062	0.006	0.017
	1979	0.024	0.019	0.013	0.046
	1980	0.023	0.019	0.011	0.017
3 + 4 (I_{3+4} + II_{st})	1976				0.163
	1977	0.090		0.174	0.235
	1978	0.150	0.161	0.147	0.124
	1979	0.203	0.188	0.095	0.138
	1980	0.153	0.126	0.105	0.107
3 + 4 + 7 (I_{3+4} + II_7)	1976				0.444
	1977	0.439		0.500	0.259
	1978	0.473	0.444	0.542	0.424
	1979	0.343	0.475	0.582	0.425
	1980	0.405	0.585	0.611	0.550
3 + 4 + 1 (I_{3+4} + II_1)	1976				0.019
	1977	0.027		0.028	0.012
	1978	0.023	0.031	0.034	0.028
	1979	0.014	0.025	0.044	0.029
	1980	0.023	0.025	0.021	0.004
3 + 4 + 2 (I_{3+4} + II_2)	1976				0
	1977	0		0	0
	1978	0.009	0.019	0.011	0.006
	1979	0.005	0.012	0	0.006
	1980	0.023	0.019	0	0.004
3 + 4 + 6 (I_{3+4} + II_6)	1976				0
	1977	0		0.014	0
	1978	0	0	0	0.006
	1979	0	0	0	0
	1980	0	0	0	0
3 + 4 + 17 (I_{3+4} + II_{17})	1976				0
	1977	0		0	0
	1978	0	0	0	0
	1979	0.005	0	0	0
	1980	0	0	0.010	0

TABLE 1—Continued

O arrangement by segments (I + II)	Year	Season[a] S	ES	LS	A
3 + 4 + 22 ($I_{3+4} + II_{22}$)	1976				0.008
	1977	0.005		0.014	0.012
	1978	0	0.025	0.011	0.017
	1979	0.014	0.031	0.025	0.023
	1980	0.015	0.025	0.032	0.008
3 + 4 + 8 ($I_{3+4+8} + II_{st}$)	1976				0.105
	1977	0.140		0.106	0.148
	1978	0.118	0.099	0.073	0.141
	1979	0.145	0.087	0.120	0.121
	1980	0.160	0.075	0.074	0.116
3 + 4 + 8 + 7 ($I_{3+4+8} + II_7$)	1976				0
	1977	0.009		0	0
	1978	0.005	0	0	0
	1979	0	0.006	0	0
	1980	0	0	0	0
3 + 4 + 8 + 1 ($I_{3+4+8} + II_1$)	1976				0.004
	1977	0		0	0
	1978	0	0	0	0
	1979	0	0	0	0
	1980	0	0	0	0
3 + 4 + 16 + 2 ($I_{3+4+16} + II_2$)	1976				0
	1977	0		0.005	0
	1978	0	0	0	0
	1979	0.005	0.006	0	0
	1980	0.004	0.006	0	0.004
3 + 4 + 13 + 2 ($I_{3+4+13} + II_2$)	1976				0
	1977	0		0	0
	1978	0	0	0.006	0
	1979	0	0	0	0
number of genomes	1976				257
	1977	221		218	81
	1978	220	162	177	177
	1979	207	160	158	174
	1980	262	159	95	242

[a] S = spring; ES = early summer; LS = late summer; A = autumn.

region of segment I (according to LOUKAS *et al.* 1979) and that this prevents recombination (except for double crossing overs) between allozymes included in different arrangements (O_{3+4} and O_{st}) of the segment I. The practical effect of this is the isolation of O_{3+4} allozymes from those of O_{st}, with the result that each set of allozymes evolves as an independent gene complex in terms of recombination.

Table 3 gives the allozyme frequencies inside each arrangement by season

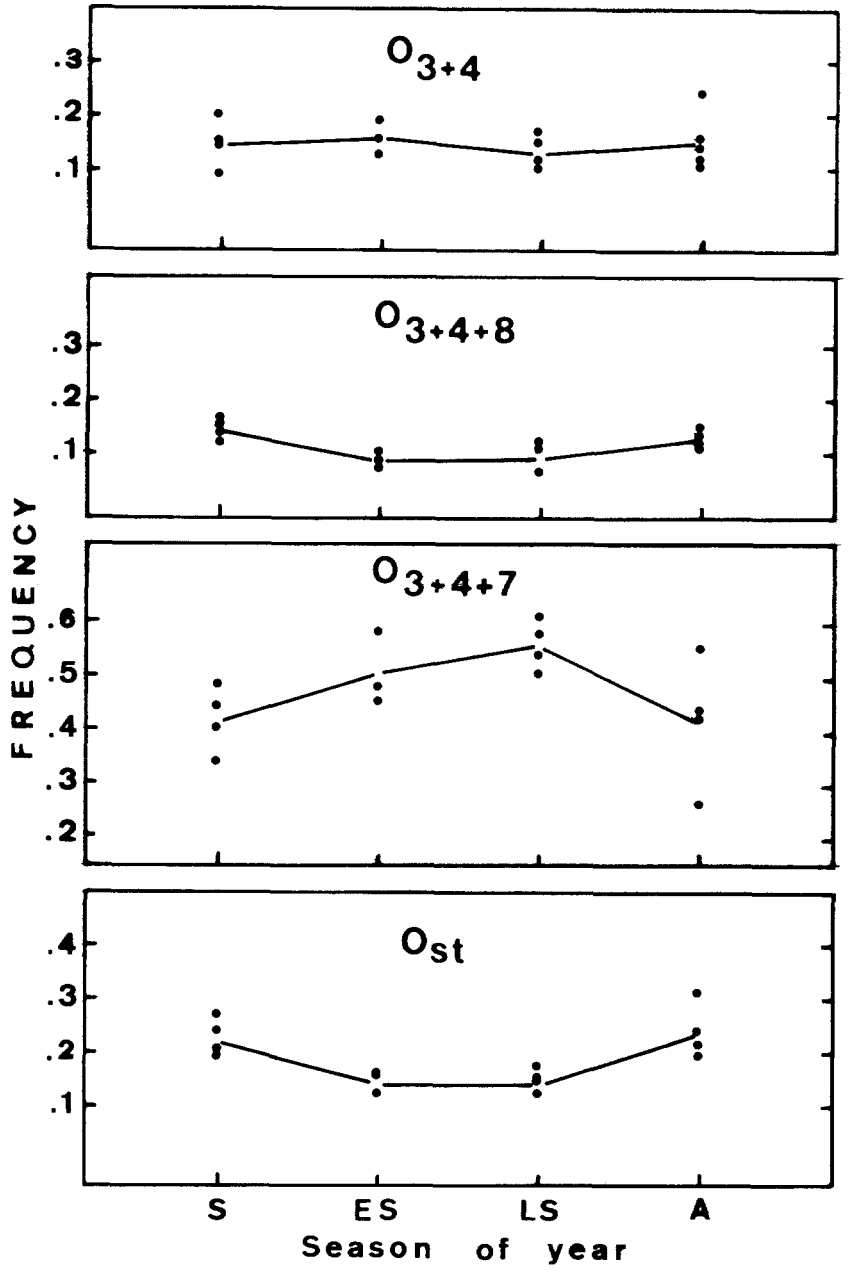


FIGURE 1.—Seasonal changes of several arrangement frequencies in the natural population of El Pedroso. Each dot represents a year sample. Straight lines units average year values of each season. S = spring; ES = early summer; LS = late summer; A = autumn.

and year. In this table, mean heterozygosities ($\bar{H}$) are also shown for each allozyme locus inside each arrangement at each sampling season. Heterozygosities for *Lap* are consistently higher inside O_{st} than inside the O_{3+4} arrangement with means of 0.557 and 0.294, respectively, which are highly different statis-

TABLE 2

Electromorph frequencies of Lap, Pept-1 and Acph loci by year and season in the natural population of El Pedroso

Electromorph	Year	Season[a] S	ES	LS	A
Lap					
1.18	1976				0.035
	1977	0.023		0.023	0.037
	1978	0.023	0.037	0.011	0.006
	1979	0.043	0.056	0.013	0.011
	1980	0.038	0.044	0.032	0.025
1.11	1976				0.198
	1977	0.163		0.170	0.222
	1978	0.150	0.043	0.164	0.220
	1979	0.184	0.106	0.196	0.218
	1980	0.176	0.164	0.179	0.202
1.00	1976				0.732
	1977	0.783		0.739	0.691
	1978	0.777	0.883	0.780	0.751
	1979	0.729	0.787	0.766	0.713
	1980	0.740	0.767	0.768	0.744
0.86	1976				0.027
	1977	0.032		0.050	0.049
	1978	0.027	0.031	0.034	0.023
	1979	0.034	0.031	0.025	0.034
	1980	0.038	0.019	0.011	0.021
0.69	1976				0.008
	1977	0		0.018	0
	1978	0.023	0.006	0.011	0
	1979	0.010	0.019	0	0.023
	1980	0.008	0.006	0.011	0.008
Pept-1					
1.60	1978		0.043	0.079	0.034
	1979	0.043	0.044	0.032	0.029
	1980	0.019	0.031	0.053	0.033
1.00	1978		0.583	0.294	0.407
	1979	0.357	0.350	0.342	0.437
	1980	0.405	0.340	0.453	0.322
0.40	1978		0.374	0.621	0.548
	1979	0.594	0.594	0.614	0.534
	1980	0.573	0.623	0.484	0.632
0.17	1978		0	0.006	0.011
	1979	0.005	0.012	0.013	0
	1980	0.004	0.006	0.011	0.012

TABLE 2—Continued

Electromorph	Year	Season[a] S	ES	LS	A
Acph					
2.00	1978			0.011	0.006
	1979	0.029	0.019	0.006	0.011
	1980	0.008	0.006	0.021	0.025
1.88	1978			0.062	0.028
	1979	0.024	0.019	0.032	0.011
	1980	0.034	0.031	0.042	0.021
1.00	1978			0.831	0.892
	1979	0.850	0.894	0.880	0.874
	1980	0.905	0.887	0.863	0.859
0.54	1978			0.090	0.068
	1979	0.087	0.069	0.082	0.103
	1980	0.053	0.075	0.074	0.091
0.25	1978			0.006	0.006
	1979	0.010	0	0	0
	1980	0	0	0	0.004

[a] Abbreviations for seasons are defined in Table 1 footnote.

tically. On the other hand, heterozygosities are not statistically different between arrangements for *Pept-1* and *Acph*.

Table 4 gives the indices of gametic disequilibrium by year and season between the electromorphs and the *O* chromosome arrangements. The gametic disequilibrium between segment I of *O* chromosome and *Lap* is highly significant in all of the sampled seasons except in the early summers of 1978 and 1980 (chi square values in Table 4). In spite of the variability of gametic disequilibrium between years in each season, there is a seasonal trend of variation. Gametic association is lowest in the early summer and increases toward the autumn (Figure 4a). Further analyses show that the associations between $Lap^{1.00}$ and O_{3+4} and between $Lap^{1.11}$ and O_{st} are those in excess. Furthermore, it is apparent that the slight mean increase in the frequency of $Lap^{1.11}$ from early summer to autumn, mentioned earlier, is exclusively caused by the changes in the $Lap^{1.11}$ frequencies within the O_{st} arrangement (Figure 2a), where there is a yearly significant increase (regression slope = 0.99, $t = 4.25$, $P < 0.01$) of $Lap^{1.11}$ from a minimum in early summer toward autumn. Figure 2a also depicts the $Lap^{1.11}$ frequencies found within the inverted O_{3+4} segment. Within the O_{3+4} arrangement, there are no statistically significant seasonal changes in the frequencies of this allozyme. The frequency of $Lap^{1.11}$ within O_{3+4} ($\sim$0.10) is significantly lower than that within O_{st} for each sampled season except in the early summer when the lowest $Lap^{1.11}$ frequencies are found.

Pept-1 and the *O* arrangements also show gametic disequilibrium which is similar to that of the *O-Lap* associations in that it is seasonally lowest in the

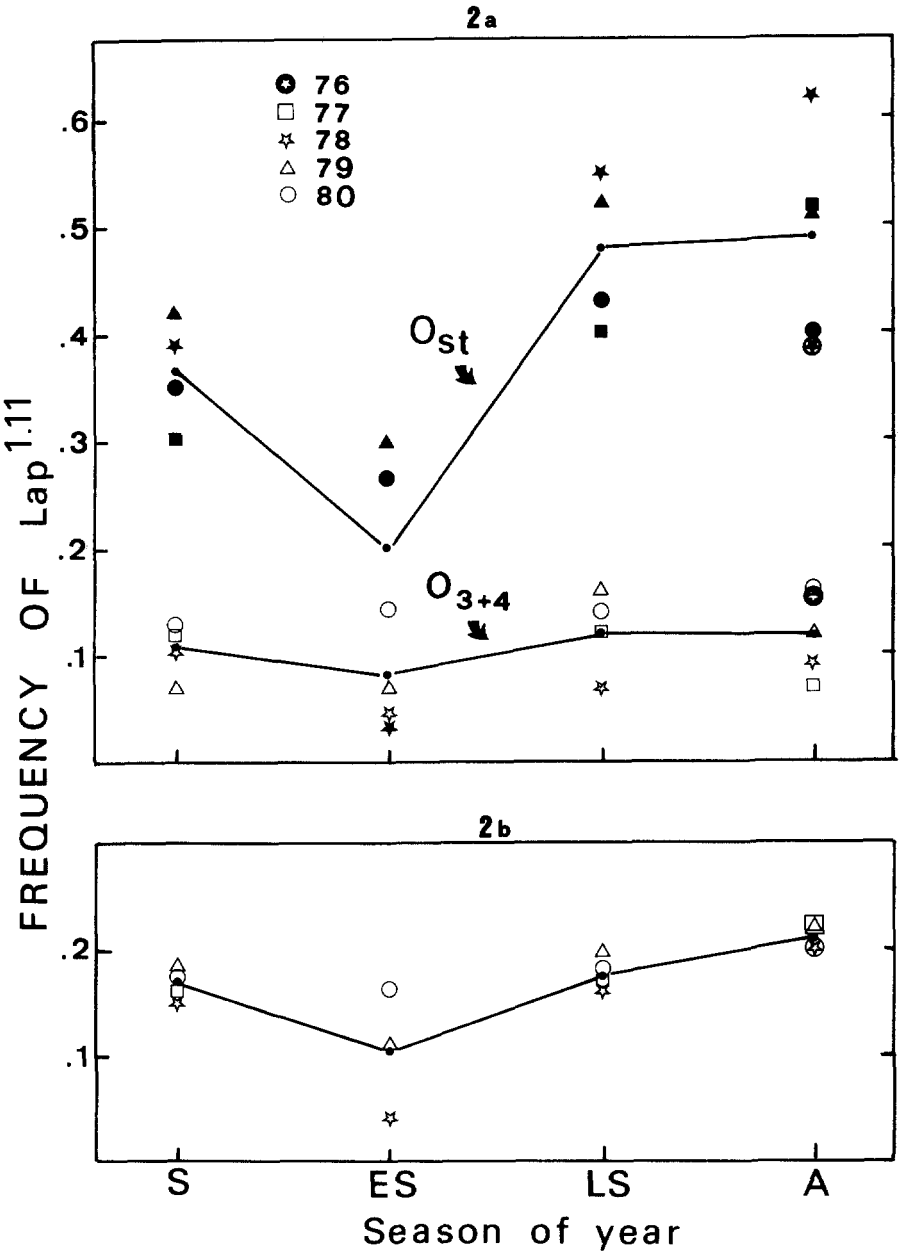


FIGURE 2.—Frequencies of $Lap^{1.11}$ electromorph by season and year. a, Seasonal frequencies inside each arrangement O_{st} and O_{3+4}; b, pooled frequencies in total year sample. Straight lines unite average year values of each season. S = spring; ES = early summer; LS = late summer; A = autumn.

early summers (Table 4; Figure 4b). All of the chi square values in Table 4 are highly significant (2×2 χ^2 test) except those of the early summers and that of the late summer of 1980. Further analyses of the *Pept-1* alleles showed that, although the overall frequency of $Pept\text{-}1^{0.40}$ does not indicate a significant

TABLE 3

Frequencies and heterozygosities by year and season of electromorphs associated with each of major arrangements of segment I of O chromosome (O_{st} and O_{3+4})[a]

Electromorph	Year	Season[a] S	ES	LS	A
Lap1.00					
Within O_{st}	1976				0.515
	1977	0.578		0.486	0.333
	1978	0.480	0.857	0.387	0.378
	1979	0.455	0.556	0.429	0.378
	1980	0.509	0.591	0.429	0.520
Within O_{3+4}	1976				0.791
	1977	0.847		0.792	0.881
	1978	0.840	0.893	0.871	0.860
	1979	0.843	0.838	0.797	0.833
	1980	0.790	0.798	0.824	0.791
Lap1.11					
Within O_{st}	1976				0.379
	1977	0.312		0.400	0.519
	1978	0.388	0.029	0.548	0.622
	1979	0.418	0.296	0.524	0.511
	1980	0.351	0.273	0.429	0.400
Within O_{3+4}	1976				0.147
	1977	0.121		0.119	0.071
	1978	0.097	0.045	0.068	0.093
	1979	0.074	0.068	0.161	0.120
	1980	0.130	0.137	0.135	0.160
Heterozygosity					
Within O_{3+4}	1976				0.351
	1977	0.267		0.355	0.218
	1978	0.283	0.206	0.234	0.250
	1979	0.281	0.289	0.338	0.291
	1980	0.356	0.343	0.302	0.348
$\bar{H} \pm$ S.E.[c]		0.297 ±0.020	0.279 ±0.040	0.307 ±0.027	0.292 ±0.026
Within O_{st}	1976				0.583
	1977	0.562		0.597	0.609
	1978	0.608	0.256	0.546	0.470
	1979	0.609	0.590	0.539	0.592
	1980	0.602	0.566	0.622	0.563
$\bar{H} \pm$ S.E.[c]		0.595 ±0.011	0.471 ±0.108	0.576 ±0.020	0.563 ±0.025
Pept-1$^{1.00}$					
Within O_{st}	1978		0.629	0.613	0.733
	1979	0.509	0.481	0.810	0.600
	1980	0.702	0.455	0.643	0.560

TABLE 3—Continued

Electromorph	Year	Season[a] S	ES	LS	A
Within O_{3+4}	1978		0.536	0.197	0.262
	1979	0.314	0.316	0.288	0.389
	1980	0.296	0.307	0.432	0.270
Pept-1$^{0.40}$					
Within O_{st}	1978		0.314	0.226	0.222
	1979	0.382	0.444	0.190	0.356
	1980	0.263	0.545	0.214	0.380
Within O_{3+4}	1978		0.420	0.735	0.692
	1979	0.653	0.632	0.661	0.593
	1980	0.685	0.645	0.541	0.687
Heterozygosity					
Within O_{3+4}	1978		0.534	0.417	0.451
	1979	0.474	0.499	0.479	0.497
	1980	0.443	0.488	0.520	0.454
$\bar{H}$ ± S.E.[c]		0.458 ±0.015	0.507 ±0.014	0.472 ±0.030	0.467 ±0.015
Within O_{st}	1978		0.503	0.547	0.411
	1979	0.586	0.566	0.308	0.511
	1980	0.437	0.496	0.520	0.538
$\bar{H}$ ± S.E.[c]		0.511 ±0.074	0.522 ±0.022	0.458 ±0.076	0.487 ±0.039
Acph$^{1.00}$					
Within O_{st}	1978			0.806	0.956
	1979	0.836	1.000	0.952	0.911
	1980	0.947	0.864	0.857	0.860
Within O_{3+4}	1978			0.818	0.888
	1979	0.851	0.872	0.856	0.843
	1980	0.889	0.887	0.865	0.853
Acph$^{0.54}$					
Within O_{st}	1978			0.097	0
	1979	0.073	0	0.048	0.067
	1980	0.018	0.136	0	0.060
Within O_{3+4}	1978			0.098	0.084
	1979	0.099	0.077	0.093	0.130
	1980	0.068	0.065	0.081	0.110
Heterozygosity					
Within O_{3+4}	1978			0.317	0.204
	1979	0.264	0.232	0.257	0.272
	1980	0.204	0.207	0.242	0.260

TABLE 3—Continued

Electromorph	Year	Season[b] S	ES	LS	A
$\bar{H}$ ± S.E.[c]		0.234 ±0.030	0.219 ±0.012	0.272 ±0.023	0.245 ±0.021
Within O_{st}	1978			0.336	0.085
	1979	0.293	0.000	0.091	0.165
	1980	0.102	0.235	0.245	0.254
$\bar{H}$ ± S.E.[c]		0.197 ±0.095	0.117	0.224 ±0.072	0.168 ±0.049

[a] $O_{st}(O_{3+4})$ includes all arrangements that are O_{st} (O_{3+4}) in segment I and show any arrangement in segment II.

[b] Abbreviations for seasons are defined in Table 1 footnote.

[c] $\bar{H}$ = mean heterozygosity.

seasonal trend (Figure 3a), it does show a significant seasonal trend within the O_{st} arrangement with high values in early summer and significantly lower values in the other seasons (Figure 3b). On the other hand, the same electromorph does not show significant changes when it is included in the O_{3+4} arrangement. The frequency of *Pept-1*$^{0.40}$ within O_{3+4} is significantly higher than that within O_{st} in each season except the early summer when it is at its lowest point.

Table 4 shows also the values of gametic disequilibrium for the *O-Acph* association. All values but one are nonsignificant (by the 2 × 2 χ^2 test), and there is no observational trend in variation through seasons. One must be aware that a possible reason for lack of significance in this association is the highly deviated frequencies of this locus from intermediate values. The mean frequency of *Acph*$^{1.00}$ computed from Table 2 is 0.873.

The analysis of gametic disequilibrium is completed by the computation of indices for *Lap* and *Pept-1* association inside each major arrangement. Figure 5 depicts the yearly trend of the correlation index (r) for illustration. The smallness of gametic sample size prevents detection of significance of any possible gametic disequilibrium, but the consistent seasonal variation observed inside the O_{st} arrangement provides a basis on which to assign significance to the association generated in early summer (Figure 5a). On the other hand, no consistent variation is observed inside the O_{3+4} arrangement, in which gametic frequencies deviate randomly to produce alternation of r values from season to season around zero. Within the O_{st} arrangement the association *Lap*$^{1.11}$-*Pept-1*$^{0.40}$ is favored in summer and disfavored in other seasons.

DISCUSSION

D. subobscura shows latitudinal variation in its chromosomal polymorphism. Such variation, together with altitudinal and especially seasonal changes, exists in *D. pseudoobscura*, and they have been taken as indications of flexible polymorphism [see DOBZHANSKY (1970) for a review]. However, seasonal and altitudinal changes are, for the most part, absent in *D. subobscura*, and the majority

TABLE 4

Indices of gametic disequilibrium by year and season between O *arrangements* (O_{st} *and* O_{3+4}) *and electromorphs at* Lap, Pept-1 *and* Acph *loci in the natural population of El Pedroso*

Indices	Year	Season[a] S	ES	LS	A
O-Lap					
χ^2	1976				17.06**
	1977	12.43**		17.23**	21.11**
	1978	24.69**	0.13 (n.s.)	43.96**	44.76**
	1979	33.03**	13.08**	14.18**	30.81**
	1980	16.04**	3.31 (n.s.)	8.51**	14.33**
D'	1976				+0.323
	1977	+0.613		+0.329	+0.719
	1978	+0.419	−0.300	+0.572	+0.617
	1979	+0.600	+0.396	+0.422	+0.497
	1980	+0.321	+0.171	+0.376	+0.271
r	1976				+0.286
	1977	+0.341		+0.317	+0.575
	1978	+0.363	−0.035	+0.536	+0.550
	1979	+0.457	+0.319	+0.328	+0.462
	1980	+0.286	+0.153	+0.317	+0.272
O-Pept-1					
χ^2	1978		1.17 (n.s.)	27.47**	30.16**
	1979	8.77**	3.06 (n.s.)	19.15**	6.60**
	1980	30.54**	1.45 (n.s.)	3.84 (n.s.)	15.53**
D'	1978		+0.194	+0.620	+0.599
	1979	+0.287	+0.241	+0.691	+0.309
	1980	+0.536	+0.184	+0.507	+0.373
r	1978		+0.091	+0.431	+0.457
	1979	+0.228	+0.150	+0.378	+0.212
	1980	+0.377	+0.103	+0.211	+0.277
O-Acph					
χ^2	1978			3.00 (n.s.)	3.96*
	1979	0.24 (n.s.)	2.34 (n.s.)	0.55 (n.s.)	1.30 (n.s.)
	1980	2.10 (n.s.)	1.20 (n.s.)	1.10 (n.s.)	0.94 (n.s.)
D'	1978			0.000	−1.000
	1979	−0.172	−1.000	−0.429	−0.416
	1980	−0.667	+0.134	−1.000	−0.348
r	1978			0.000	−0.164
	1979	−0.037	−0.133	−0.057	−0.097
	1980	−0.098	+0.092	−0.120	−0.063

[a] Abbreviations for seasons are defined in Table 1 footnote.
* $P < 0.05$; ** $P < 0.01$; n.s. = nonsignificant.

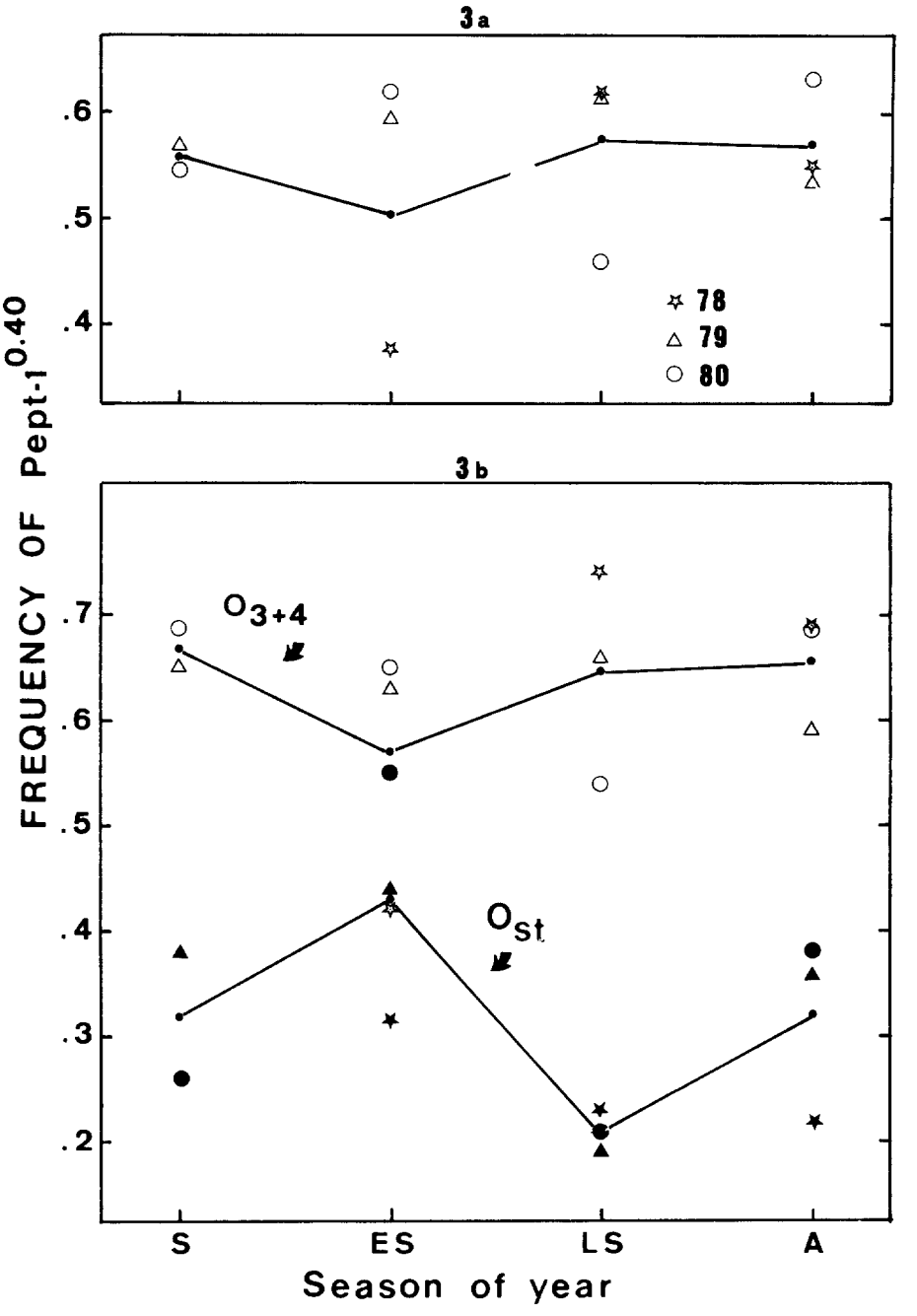


FIGURE 3.—Frequencies of *Pept* $1^{0.40}$ electromorph by season and year. a, Pooled frequencies in total year sample; b, frequencies inside each arrangement O_{st} and O_{3+4}. Straight lines unite average year values of each season. S = spring; ES = early summer; LS = late summer; A = autumn.

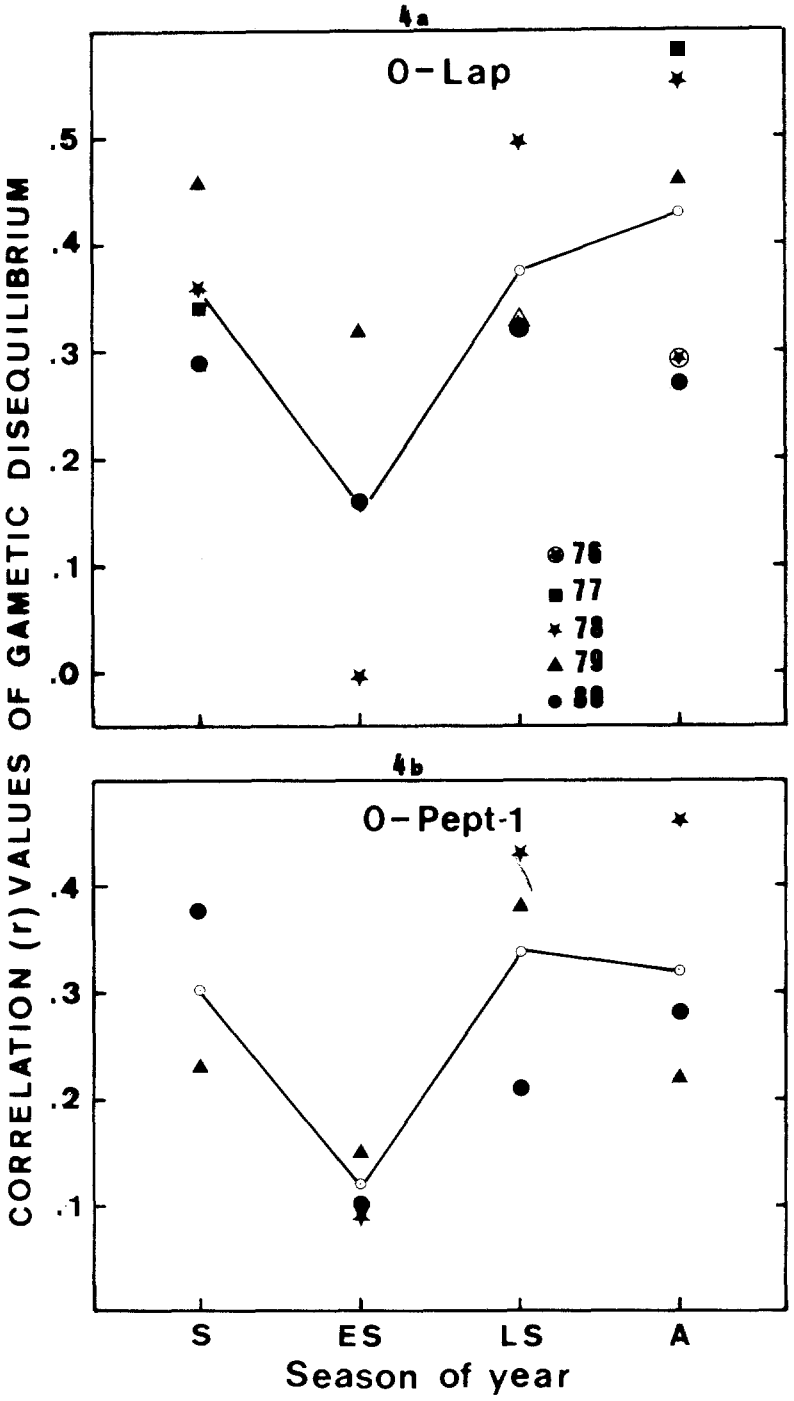


FIGURE 4.—Values of gametic disequilibrium between arrangements and electromorphs as shown by the genetic correlation (r), by season and year. a, r values for association between *O* arrangements and *Lap* electromorphs; b, r values for association between *O* arrangements and *Pept-1* electromorphs. Straight lines unite average year values of each season. S = spring; ES = early summer; LS = late summer; A = autumn.

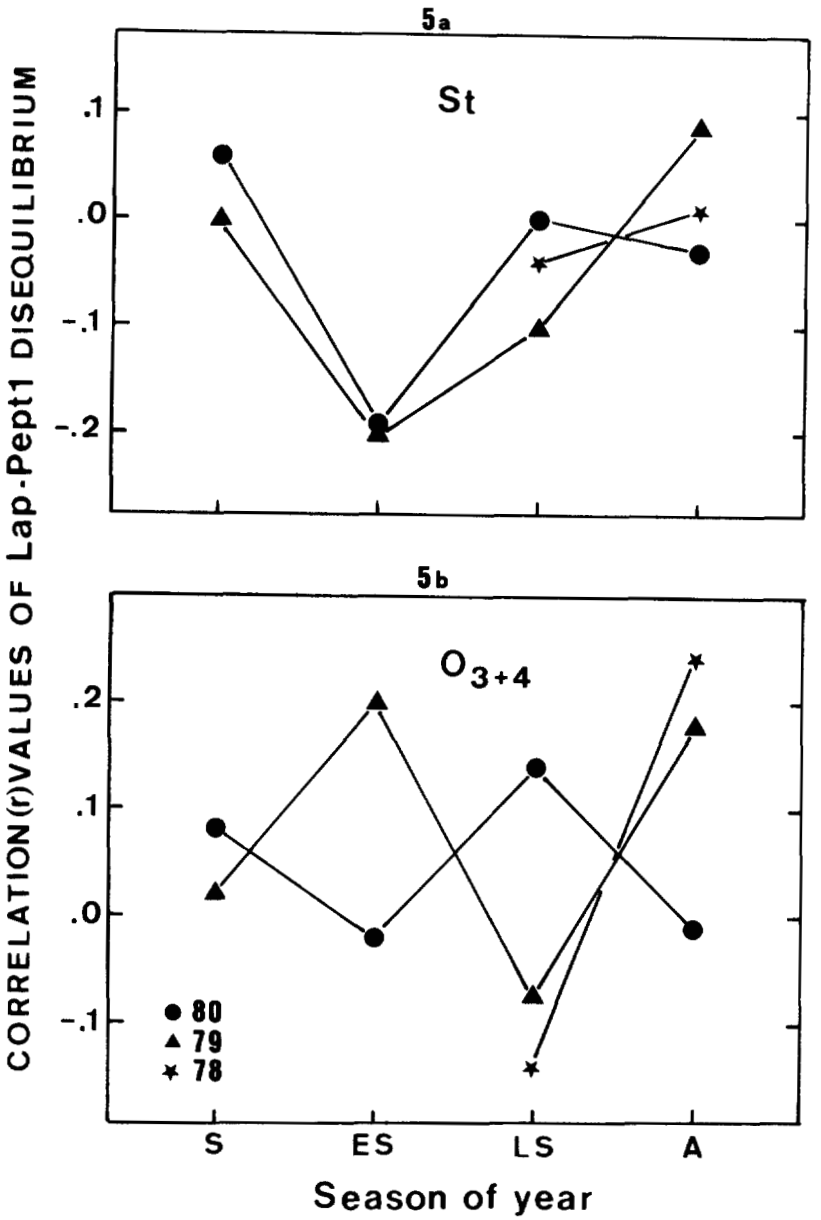


FIGURE 5.—Values of gametic disequilibrium between *Lap* and *Pept-1* loci as shown by the genetic correlation (r). a, Association inside O_{st} arrangement; b, association inside O_{3+4} arrangement. Straight lines unite actual R year values of each season. S = spring; ES = early summer; LS = late summer; A = autumn.

of the workers have concluded that this species shows a rigid chromosomal polymorphism [see KRIMBAS and LOUKAS (1980) for a review], although a few exceptions exist (BURLA and GÖTZ 1965).

The data presented in this paper demonstrate that the frequencies of O_{st} and

O_{3+4+7} change seasonally in the population of El Pedroso, whereas arrangements O_{3+4+8} and O_{3+4} do not. The reason why most workers failed to find seasonality may be because O_{3+4+7} is rare in most populations. However, this reason does not apply to the work of DE FRUTOS (1975), who did not find evidence of seasonal variation of *O* arrangements in a population in which O_{3+4+7} was in high frequency. Seasonality has always been taken as evidence of flexible polymorphism. Therefore, the discovery of seasonal variability in the chromosomal polymorphism at El Pedroso shows that a flexible polymorphism is present. Moreover, the presence of a seasonal gametic disequilibrium between the gene arrangements and the allozymes allows us to examine the process by which this polymorphism contributes to natural adaptation.

Gametic disequilibrium between an inversion system and the genes included within it does not necessarily represent proof of coadaptation but may only indicate a transient phase of decaying of the absolute gametic disequilibrium generated at the moment when the inversion was produced. This historical interpretation of the widespread gametic disequilibrium found between *O* arrangements and allozyme loci (*Lap, Pept-1*) has been the main alternative to coadaptation. This historical explanation does not work in our situation in which the differential seasonal response of the same electromorph in each arrangement must mean, for example, that there are different O_{st} supergenes, each one of which has a different relative selective value in the different seasons. This coadaptation is obviously not of the geographical type since it occurs in a single population. Several authors have failed to demonstrate in *D. subobscura* either ecological adaptation (PREVOSTI 1972; BURLA and BÄCHLI 1978; KRIMBAS and LOUKAS 1980) or geographical coadaptation (MCFARQUHAR and ROBERTSON 1963; PENTZOS-DAPONTE and SPERLICH 1965), although PREVOSTI (1957) using more favorable material found some evidence of coadaptation. Many of these data have been obtained using inappropriate material, as KRIMBAS and LOUKAS (1980) have pointed out, but they are not in disagreement with our results. In fact, our data provide the first unequivocal evidence of coadaptation in Drosophila at the level of fitness interaction in chromosomal arrangements.

Several nonselective reasons can be postulated to explain this seasonal mechanism, but they can be easily ruled out. The effect of linkage is probably not important because in the O_{st} arrangement the effective distance of recombination (EDR) between *Lap* and *Pept-1* is large (EDR ranges from 14.6 to 5.8 cM), and recombination is probably operating at a high rate in the El Pedroso population. Moreover, *Acph* is close to *Lap* and does not show association with this locus. Probably, recombination is responsible for the fast decay of disequilibrium in seasons of the year other than early summer. The central gene hypothesis can also be discarded since, as LOUKAS, KRIMBAS and VERGINI (1979) have already shown, *Acph* is not in the middle of segment I of the O_{st} arrangement and, therefore, this does not explain its lack of association.

In summary, historical and drift (or founder) effects can also be discarded because of the observed seasonality of the disequilibrium. In fact, seasonal variation of gametic disequilibrium proclaims strongly in favor of selection for different O_{st} supergenes in each season. Whether or not selection is operating

directly on the allozyme loci is a recurrent question always difficult to answer. In our case *Lap* and *Pept-1* may well be genetic markers of genetic zones, but, at least in the case of *Lap*, this zone must be small since *Acph* is closely linked to *Lap* and does not show any kind of association with it.

Two contrasting hypotheses have been put forth in order to explain the historical evolution of this chromosomal polymorphism. The first postulates that O_{st} is the primitive arrangement because there is more genetic variability in it (PREVOSTI 1978). The rationale of this is that the O_{3+4} arrangement would have captured a single allele at each locus at the moment of being produced. Later, by mutation and double recombination, the variability would be increased. However, the new arrangement would still be in the process of restoring this variability at the present moment, thus, its low observed heterozygosity. The evidence of this is based mainly on data for *Lap*, *Pept-1* and *Acph*. However they show that, although the heterozygosity of *Lap* is much higher in O_{st} than in O_{3+4}, both heterozygosities are similar for *Pept-1*, and the reverse is true for *Acph*. The second hypothesis, which postulates that O_{3+4} is primitive, is based on several facts. First, the historical evidence (PREVOSTI 1971, 1972) indicates that O_{3+4} is abundant in either relictual (Canary Islands) or ancient geographic zones (Mediterranean area), whereas O_{st} occupies the recent colonized zones of Central and Northern Europe, after the last glaciations. Also, O_{3+4} is central in the chromosome phylogeny, and this has been taken as a criterion of primitiveness (PREVOSTI 1972; OLVERA *et al.* 1979). Recently, some of us (ZAPATA, SANTOS and ALVAREZ 1982) have developed a new method to establish chromosomal phylogenies based on a modification of WALLACE's (1953) rule of triads. By using this method, we have proposed a tentative chromosomal phylogeny for *D. subobscura* in which the O_{3+4} arrangement in segment I of chromosome *O* can be inferred as the primitive one, in agreement with the earlier discussed criterion on primitiveness.

The seasonal data on gametic disequilibrium also favor the primitiveness of O_{3+4}, because O_{st} appears as the coadapted evolved complex. A tentative historical explanation would postulate that O_{3+4}, the primitive arrangement, represents a highly rigid gene ordination adapted mostly to the warm and stable climatic conditions of the Mediterranean area, the center of the species distribution. Whether this adaptation is generated either by genotypic or phenotypic adaptation is unknown. On the other hand, O_{st} appears to be a genotypically flexible arrangement that consists of a series of gene ordinations, differentially adapted to the successive seasonal environments encountered by *D. subobscura*-colonized populations in temperate paleoartic European areas. The key factors operating by natural selection may not be the usual ones (*e.g.*, temperature) but others related more to the population growth conditions such as r- and K-selection, which have been investigated by some of us (C. ZAPATA, unpublished results).

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