NATURAL SELECTION WITH NUCLEAR AND CYTOPLASMIC TRANSMISSION. II. TESTS WITH DROSOPHILA FROM DIVERSE POPULATIONS

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ABSTRACT

Observations of intraspecific variation in organelle DNA have prompted a renewed interest in the evolutionary consequences of cytoplasmically transmitted factors. Attempts to quantify the significance of cytoplasmic effects are frequently limited by the difficulty in partitioning the cause of reciprocal cross differences among a series of possibilities. In the experiment reported here the nuclear genomes of a set of six lines of *Drosophila melanogaster* from diverse geographic locations were replaced in a series of cytoplasms. The segregation of the *SM5* balancer chromsome was scored in a factorial design, and the data allowed a partitioning of variance such that cytoplasmic effects were distinguished from maternal effects and meiotic drive. An attempt was made to avoid the confounding problem of hybrid dysgenesis by performing the entire experiment (including chromosomal extractions) in a *P* cytotype. Results indicated a significant contribution of cytoplasm to the variance in *SM5* segregation. Error variance showed an increasing trend as the experiment proceeded, and additional tests indicated that this was due to an accumulation of chromosomal mutations. These findings are interpreted in light of the population genetic theory that addresses the maintenance of cytoplasmic polymorphism.

VARIATION in cytoplasmic factors provides a unique opportunity to study the evolution of traits showing non-Mendelian inheritance. The most extensively quantified class of cytoplasmically transmitted variation is mitochondrial DNA, in which polymorphism has been found in Drosophila (SHAH and LANGLEY 1979; POWELL 1983), sheep and goats (UPHOLT and DAWID 1977), mice (LANSMAN *et al.* 1981; FERRIS *et al.* 1983), pocket gophers (AVISE *et al.* 1979), rats (BROWN and SIMPSON 1982), marine mammals (S. D. FERRIS, personal communication), freshwater fishes (AVISE and SAUNDERS 1984), humans (BROWN 1980; AQUADRO and GREENBERG 1983; CANN, BROWN and WILSON 1984) and other primates (FERRIS, WILSON and BROWN 1981; BROWN, PRAGER and WILSON 1982). The chloroplast genome is approximately ten times the size of the mitochondrial genome, and although cpDNA polymorphism has been found in a number of species, it is apparently more conservative in its rate of sequence divergence (CURTIS and CLEGG 1984; CLEGG, RAWSON and THOMAS 1984).

An explanation for the level of organelle DNA polymorphism can be sought in the forces that affect nuclear gene diversity. The determination of the significance of selection and drift on organelle DNA polymorphism is made difficult by the complex way in which drift operates in cytoplasmic organelles. The theoretical dynamics of neutral organelle DNA variation have been described by TAKAHATA and MARUYAMA (1981); TAKAHATA and SLATKIN 1983; BIRKY, MARUYAMA and FUERST (1983); CHAPMAN *et al.* (1982) and AVISE, NEIGEL and ARNOLD (1984). Generally, these results show that the efficacy of drift in maintaining mtDNA diversity depends on the total population size, the number of cell divisions per organismal generation, the sex ratio, the number of mitochondria transmitted to the zygote by the mother and the father and the mutation rate. Results from models that allow selective differences among cytoplasmic factors indicate that a stringent set of requirements must be met in order to maintain a cytoplasmic polymorphism by natural selection (GREGORIUS and ROSS 1984; CLARK 1984). This may suggest that the bulk of the observed variation is either neutral or in mutation-selection balance.

Experimental data can address the selective neutrality of mtDNA variation either through directly assessing phenotypic effects or through evidence concerning the nature of the sequence variation. AQUADRO and GREENBERG (1983), FERRIS *et al.* (1983) and WOLSTENHOLME and CLARY (1985) have used sequence analysis of human, mouse and Drosophila mtDNA to reveal clear heterogeneity in the distribution of polymorphic sites. Nontranscribed regions are the most polymorphic, and within protein-coding genes, silent variation is significantly more frequent than the neutral expectation. Although these results suggest that there are evolutionary constraints to organelle DNA sequences, they do not directly address the adaptive significance of currently extant organelle DNA variation.

The neutral theory provides an elegant null hypothesis that can be used to generate expected patterns of variation in space and time (KIMURA 1983). By statistically testing the goodness-of-fit of observed data to these expected patterns, one can unequivocally determine whether a particular neutral model should be rejected. A problem arises when the neutral formulation is rejected, because rejection of the neutral model does not necessarily imply that the variation is adaptively significant. Rather, rejection may be due to improper parameterization of the neutral model.

Another approach is to formulate a selective model and attempt to estimate the parameters of this model. The null hypothesis is still taken to be an absence of selective differences, but now the focus is shifted from an observation of genotypic patterns to an assessment of phenotypic effects. Disadvantages of this approach include the necessity of working in the laboratory environment and perturbation of the genetic background, but the advantage in examining the effects of the variation at the phenotypic level allows more direct tests of selective models. In this study *Drosophila melanogaster* from six diverse geographic regions were crossed in a factorial design to generate all possible combinations of nuclear genomes and cytoplasms. The scoring of segregation

and fecundity then allowed bounds to be placed on the significance of cytoplasmic variation on components of fitness.

MATERIALS AND METHODS

Strains: The first six of the following stocks of *D. melanogaster* were used as sources of cytoplasms and second chromosomes: (1) AH198S—An isofemale line from Apple Hill, California, collected in Fall 1981 by J. COYNE. This stock had the eagle (*eg* 3-47.0) mutation. (2) DV92S—An isofemale line collected in Spring 1980 at Furnace Creek, Death Valley, California. (3) Egaa1—An isofemale line collected in Egaa, Denmark, in August 1982 by A. CLARK. (4) GB8S—An isofemale line collected at the Gundlach-Bundschu winery in northern California in April 1980 by J. COYNE. This line also had the *eg* mutation. (5) R1—An isofemale line collected in the Rothrock State Forest, Centre County, Pennsylvania, in July 1983 by A. CLARK. (6) St5—An isofemale line collected in Stillwater, Minnesota, and kindly provided by J. CURTSINGER. (7) *Bl* L^2/*SM5*—A laboratory stock bearing the multiply inverted balancer second chromosome *SM5*, characterized by the Curly wing phenotype. The Bristle (*Bl*) and Lobe (L^2) dominant markers were not used. (8) Harwich—An isofemale line collected in Harwich, Massachusetts, by M. TRACEY, JR. in 1967. This line bears a strong *P* cytotype and is useful in testing the cytotype with respect to the *P-M* system of hybrid dysgenesis. This stock was kindly provided by M. KIDWELL. (9) Canton-S—A laboratory stock used as a standard *M* cytotype.

Construction of Bl L^2/*SM5 with P cytotype*: A balancer stock with the *P* cytotype was necessary in order to extract second chromosomes from the above isofemale lines in their native cytoplasms without inducing hybrid dysgenesis. Harwich females were crossed with *Bl* L^2/*SM5* males, and virgin +/*SM5* female offspring were collected. These females had the *P* cytotype, and they were crossed to *Bl* L^2/*SM5* males. The resultant *Bl* L^2/*Sm5* progeny were sib-mated for ten generations, and cytotype testing was done by scoring gonadal dysgenesis in F_1 hybrids with Harwich and Canton-S following the procedure of SCHAEFER, KIDWELL and FAUSTO-STERLING (1979). The strain proved to have a *P* cytotype and is designated *Bl* L^2/*SM5 (P)*. Linkage tests verified that the multiply inverted chromosome was intact.

Extraction of second chromosomes: After verifying that the six isofemale lines had a *P* cytotype, second chromosomes were extracted in the cytoplasm of the respective isofemale line. For example, AH198S virgin females were mated with *Bl* L^2/*SM5 (P)* males, and their +/*SM5* virgin female offspring were crossed individually to *Bl* L^2/*SM5 (P)* males. Resulting +/*SM5* offspring of both sexes had the same wild-type second chromosome, and they were crossed to test for recessive lethality. One nonlethal second chromosome derived from each isofemale line was selected for further study. The genetic background was replaced by ten generations of repeated backcrossing to *Bl* L^2/*SM5* males. The resultant strains had a unique wild second chromosome in the original cytoplasm, with the rest of the genetic background replaced by the *Bl* L^2/*SM5 (P)* strain. Initially, the six isofemale lines showed electromorphic variation at the *Pgd* (*1*-0.6), *Pgm* (*3*-43.4), *Est-C* (*3*-47.7) and *Est-6* (*3*-36.8) loci, detected by cellulose acetate electrophoresis (S. EASTEAL and I. A. BOUSSY, unpublished results). After the replacement backcrossing, 40 individuals of each line were found to be isomorphic with the *Bl* L^2/*SM5 (P)* line.

Cytoplasmic exchange crosses: Using the notation $+_j$/*SM5* (*i*) to represent a fly bearing the *j*th second chromosome in the *i*th cytoplasm, the following crosses were performed for all six second chromosomes and cytoplasms. The first cross was $+_i$/*SM5* (*i*) females with $+_j/+_j$ (*j*) males. Curly female offspring had the cytogenotype $+_j$/*SM5* (*i*), and they were mated with $+_j/+_j$ (*j*) males. Offspring from this cross were either $+_j$/*SM5* (*i*) or $+_j/+_j$ (*i*), and they were used to establish the line with the *j*th chromosome in the *i*th cytoplasm. This crossing scheme was repeated for all chromosome-cytoplasm combinations, resulting in 36 lines. These lines were maintained by mass mating of *Cy* and wild phenotypes in bottle cultures.

Segregation tests: For each of the 36 combinations of chromosome *i* and cytoplasm *j* the following two crosses were performed: reciprocal 1: $+_i$/*SM5* (*j*) × $+_i/+_i$ (*j*) and reciprocal 2: $+_i/+_i$ (*j*) × $+_i$/*SM5* (*j*). Two virgin females and two virgin males, aged 3–4 days were placed in 95-mm shell vials. Mating and egg laying were allowed for 4 days, and the adult flies were transferred to fresh vials. These vials are referred to as duplicates. Flies were removed from the second vial after 4

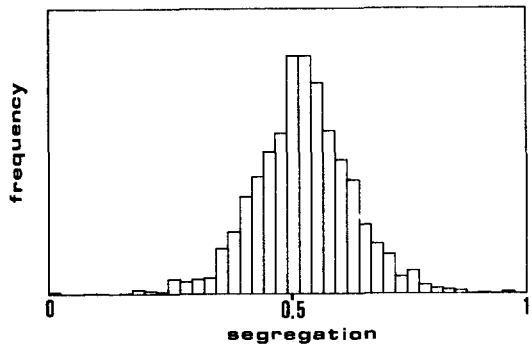

FIGURE 1.—Segregation ratio distribution from raw data (1728 vials). The segregation parameter is the fraction of wild-type progeny, with HALDANE'S (1956) bias correction.

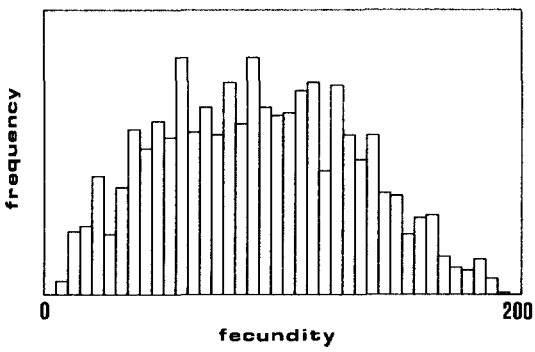

FIGURE 2.—Raw fecundity distribution. Fecundity is defined as the number of adult offspring per vial.

days of egg laying, and adult progeny were scored by sex and wing phenotype on the 17th and 18th day after egg laying began. For each of the above crosses, 12 replicate pairs of duplicates were scored. Segregation is reported as the number of wild progeny divided by one plus the total count of progeny (HALDANE 1956), and fecundity is scored as the total count of adult progeny. An attempt was made to set up four rounds of three replicates each, but this was not strictly followed, therefore, the experiment is not perfectly balanced in time. Stocks and testcrossess were maintained on Carolina 4-24 medium in incubators at 25°. Cytotype testcrosses and rearing were performed at 29°.

RESULTS

A total of 154,339 flies were scored in the segregation study, and they fell into the data structure: six chromosomes × six cytoplasms × two reciprocals × 12 replicates × two duplicates × two sexes × two phenotypes (*Cy* and wild). The data were balanced with respect to the numbers of replicates and duplicates, simplifying the analyses. The distributions of segregation and fecundity appear in Figures 1 and 2. The mean segregation parameter was 0.532, and the mean fecundity was 89.32 flies/vial.

Figures 3, 4, and 5 are scattergrams that reveal the extent to which the repeated backcrossing removed sex chromosome variation. Figure 3 shows that the duplicate pairs of vials are not correlated ($r = 0.114$, $P > 0.05$) in sex

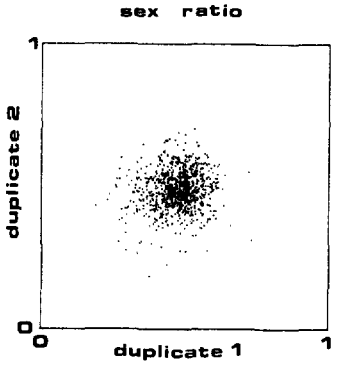

FIGURE 3.—Scattergram of raw sex ratio (fraction male) data. The lack of correlation suggests a lack of *X*-linked variation.

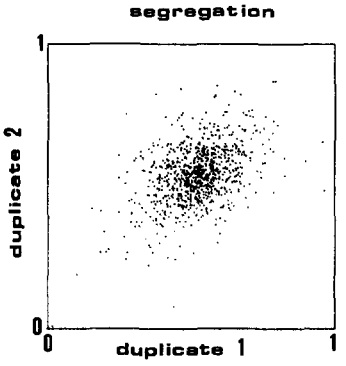

FIGURE 4.—Segregation of second chromosomes in the duplicate pairs are correlated, indicating genetic variation.

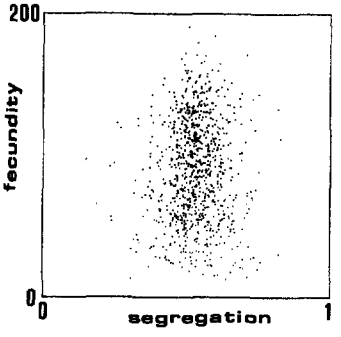

FIGURE 5.—Fecundity and segregation of second chromosomes are not correlated.

ratio, suggesting that there was no significant genetic variation in sex chromosome segregation or sex-specific viability. This serves as further evidence that the ten generations of replacement backcrossing successfully removed *X* chromosome variation. Figure 4 reveals a significant correlation between duplicates in second chromosome segregation ($r = 0.431$, $P < 0.001$), consistent

TABLE 1

Analysis of variance of segregation data

Source	d.f.	MS	Variance (÷100)	% variance	Expected MS
N	5	0.0621*	0.0305 ± 0.0233	3.97	$2\sigma^2_{NCB} + 12\sigma^2_{NB} + 24\sigma^2_{NC} + 144\sigma^2_{N}$
C	5	0.0345	0.0096 ± 0.0133	1.25	$2\sigma^2_{NCB} + 12\sigma^2_{CB} + 24\sigma^2_{NC} + 144\sigma^2_{C}$
R	1	0.0407			$\sigma^2_{NCRB} + 6\sigma^2_{CRB} + 6\sigma^2_{NRB} + 12\sigma^2_{NCR} + 36\sigma^2_{RB} + 72\sigma^2_{CR} + 72\sigma^2_{NR} + 432\Sigma\rho^2$
B	11	0.0098	−0.0011 ± 0.0063	0	$2\sigma^2_{NCB} + 12\sigma^2_{CB} + 12\sigma^2_{NB} + 72\sigma^2_{B}$
NC	25	0.0174***	0.0449 ± 0.0199	5.82	$2\sigma^2_{NCB} + 24\sigma^2_{NC}$
NR	5	0.0130	0.0100 ± 0.0101	1.30	$\sigma^2_{NCRB} + 6\sigma^2_{NRB} + 12\sigma^2_{NCR} + 72\sigma^2_{NR}$
NB	55	0.0075	0.0066 ± 0.0125	0.86	$2\sigma^2_{NCB} + 12\sigma^2_{NB}$
CR	5	0.0046	−0.0028 ± 0.0046	0	$\sigma^2_{NCRB} + 6\sigma^2_{CRB} + 12\sigma^2_{NCR} + 72\sigma^2_{CR}$
CB	55	0.0099*	0.0268 ± 0.0161	3.46	$2\sigma^2_{NCB} + 12\sigma^2_{CB}$
RB	11	0.0063	0.0021 ± 0.0082	0.27	$\sigma^2_{NCRB} + 6\sigma^2_{CRB} + 6\sigma^2_{NRB} + 36\sigma^2_{RB}$
NCR	25	0.0065	0.0038 ± 0.0153	0.48	$\sigma^2_{NCRB} + 12\sigma^2_{NCR}$
NCB	275	0.0067	0.0319 ± 0.0381	4.12	$2\sigma^2_{NCB}$
NRB	55	0.0053	−0.0118 ± 0.0186	0	$\sigma^2_{NCRB} + 6\sigma^2_{NRB}$
CRB	55	0.0062	0.0032 ± 0.0212	0.42	$\sigma^2_{NCRB} + 6\sigma^2_{CRB}$
NCRB	275	0.0060	0.6030 ± 0.0722	78.07	σ^2_{NCRB}

Significance of mean squares (MS) was determined by appropriate F statistics. Variance components were determined by equating mean squares to expected mean squares, and standard errors were determined from the theoretical sampling estimates. N, nucleus; C, cytoplasm; R, reciprocal; B, block.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

with previous reports of variation in segregation among extracted chromosomes. Figure 5 shows that fecundities and segregations are not correlated ($r = 0.019$, $P > 0.05$), allowing independent analyses of these components. In all of the analyses that follow, duplicates were pooled.

Analysis of segregation—linear model: The experimental design is unusual in that cytoplasms and reciprocal crosses are orthogonal variables. This allows the use of the following linear model:

$$s_{ijkl} = u + n_i + c_j + r_k + nc_{ij} + nr_{ik} + cr_{jk} + ncr_{ijk} + e_{ijkl}$$

where s_{ijkl} is the segregation parameter of the ith chromosome in the jth cytoplasm of the kth reciprocal of the lth replicate. The terms n_i, c_j and r_k refer to the nuclear (chromosomal), cytoplasmic and reciprocal cross effects, respectively, and other terms represent the hierarchical interactions. e_{ijkl} is the error term, assumed to be normally distributed with mean zero. Chromosomes and cytoplasms are treated as random effects in the model, whereas reciprocal is a fixed effect. Means and variances of segregations were found to be uncorrelated, but the error variance showed significant departure from constancy. Since replicates were scored at different times, the significance of time as an experimental factor was considered by treating replicates as "blocks" and including it as another random treatment effect.

Analysis of variance of the resulting saturated linear model is presented in Table 1. Variance components were estimated by equating the mean squares

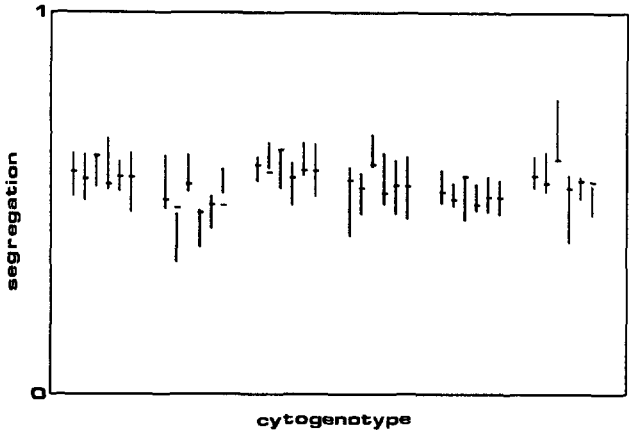

FIGURE 6.—Summary of segregation data. Error bars represent means ±2 SE in segregation parameters for each chromosome and cytoplasm combination. The first six bars represent the AH198S chromosome in six cytoplasms (in alphabetical order). Successive groups of six bars represent the successive chromosomes in all cytoplasms, again in alphabetical order. Horizontal dashes show predictions of the model with no additive interaction between chromosomes and cytoplasms.

to the expected mean squares and solving the resulting system of linear equations. Standard errors of variance components were estimated using normal theory (SEARLE 1971). Negative variance components may represent some departure from the model, but negative values were uniformly small, and they were equated to zero in calculating the percentage variance components. Analysis of the residuals of the linear model showed an excellent fit to a normal distribution, and plots of predicted *vs.* observed values showed no trends. The results were remarkably robust with respect to both logarithmic and angular transformation, and reported mean squares are from the untransformed data.

The significant nuclear effect seen in the analysis presented in Table 1 was expected based on prior observation of segregation variation among extracted second chromosomes. Cytoplasms did not appear as a significant main effect, but the nuclear × cytoplasmic interaction was surprisingly large. The meaning of this interaction can be explored by examining the segregation values plotted in Figure 6. Some second chromosomes, such as in AH198S and R1, have a segregation ratio that is independent of the cytoplasmic origin, whereas others, such as in DV92S, yield segregation ratios that are greatly perturbed by different cytoplasms. Although the biological mechanism for this interaction has not yet been elucidated, it is evidently not a meiotic effect because reciprocal crosses gave similar segregation ratios. The lack of a cytoplasmic main effect evidently implies that the cytoplasms do not exert a common influence on the *SM5* to wild segregation.

One particular question that can be answered by Figure 6 is whether the *i*th chromosome is at an advantage in the *i*th cytoplasm. Plotted in Figure 6 are the expected means based on a model that assumes an additive action of nuclear and cytoplasmic effects. These expectations were determined from

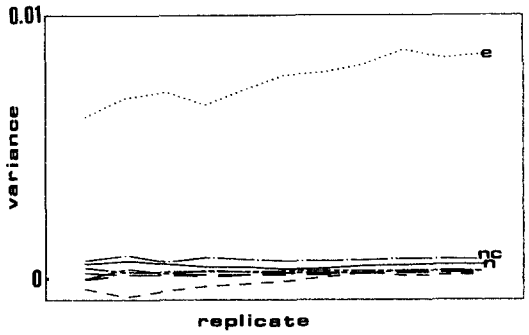

FIGURE 7.—Variance components of segregation data calculated from cumulative sets of replicates. Nuclear, nuclear × cytoplasm and error components are indicated by letters.

predicted values of a linear model that do not allow a nuclear × cytoplasmic interaction. The results clearly fail to demonstrate any coadaptation between chromosomes and cytoplasms, and in two of the six cases an extracted chromosome was at a disadvantage in its native cytoplasm.

To further explore the consequences of the temporal variation, the analysis of variance was repeated taking cumulative sets of replicates from replicate one to replicate "i," where i was from 2–12. When components of variance are calculated for these analyses of variance (Figure 7), an increasing trend in error variance becomes evident, but the other factors do not display this trend. The nuclear effect and the nuclear × cytoplasm interaction remain consistent in their significant contribution to the total variance, indicating that this result is robust with respect to the temporal heterogeneity. The increasing error variance may be a real biological phenomenon, due either to an accumulation of nuclear genetic mutations or to cytoplasmic instability. These possibilities were tested experimentally by reextracting chromosomes from two of the stocks used in the factorial experiment 32 generations after the initial extraction. Chromosomes were extracted using the *Bl* L^2*/SM5* balancer stock in such a way that reextracted lines either had the same maternal lineage (hence, having a common "reextracted" cytoplasm) or the same second chromosome in a set of maternal lineages. Results are shown in Figures 8 and 9. One-way analysis of variance indicates that, when a single chromosome is reextracted in a series of maternal lines, the segregation ratios are homogeneous ($F_{9,131}$ = 0.48, not significant (NS) for line DE, and $F_{7,109}$ = 1.03, NS for line ER). When several second chromosomes are reextracted from a single stock, however, the segregation ratios are significantly heterogeneous, indicating an accumulation of mutations affecting viability ($F_{7,102}$ = 2.27, $P < 0.05$ for line DE, and $F_{6,77}$ = 2.34, $P < 0.05$ for line ER). These results are analogous to MUKAI's (1964) results and indicate that second chromosome mutations accumulate in stocks even if they are not kept balanced by *SM5* every generation.

Analysis of segregation—log-linear models: Although the data fitted the linear model quite well, counted data of the sort collected here lend themselves to powerful discrete multifactorial analyses. Log-linear analysis is particularly useful in this context, because it is easier to interpret sex as a response variable

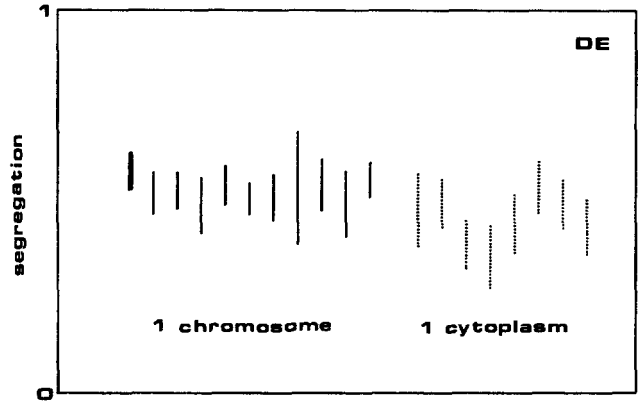

FIGURE 8.—Segregations (±2 SE) of second chromosomes from reextractions performed after the conclusion of the factorial experiments. On the left are reextractions of a single second chromosome from the DV92S line with the Egaa1 cytoplasm in a series of maternal lines. On the right is a series of reextracted second chromosomes within a common maternal line.

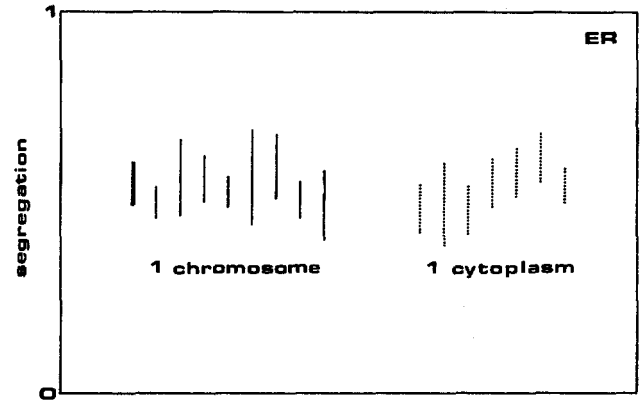

FIGURE 9.—Segregations of reextracted Egaa1 chromosomes in the R1 cytoplasm formatted as in Figure 6.

than in the analogous multivariate linear model. When a log-linear analysis is applied to these data, all of the design variables (nucleus, cytoplasm, reciprocal and replicate = block) are retained in a "fixed configuration" (BISHOP 1969; BISHOP, FIENBERG and HOLLAND 1975). The response variables, phenotype and sex, can then interact with each other as well as any hierarchical subset of the design variables. To apply log-linear models, we must first account for the sampling distribution. The sampling of many flies from each vial results in a form of nonindependence called "cluster sampling" (ALTHAM 1976) and yields a Dirichlet-multinomial distribution (BRIER 1980). MOSIMANN (1962) showed that the variance-covariance matrix of the Dirichlet-multinomial is simply a constant multiplied by the variance-covariance matrix of the founding multinomial distribution. This fact allows the application of log-linear models after scaling the resultant chi squares by this constant. BRIER (1980) showed that the constant can be estimated by $C = (\chi^2_{het}/df)$ or the heterogeneity chi

TABLE 2

Log-linear analysis of segregation

Term	G^2/C	d.f.	P
NC	161.64	25	0.0002
N	68.58	5	0.0003
C	66.49	5	0.0004
NCR	62.00	25	0.0007
S	10.74	1	0.0009
R	10.68	1	0.0009
NR	27.59	5	0.0136
CR	10.86	5	0.0544
NS	5.98	5	0.3082
RS	0.50	1	0.4793
CRS	2.98	5	0.7036
NRS	1.58	5	0.9037
CS	1.02	5	0.9610
NCRS	11.33	25	0.9912
NCS	10.87	25	0.9936

In rank order of probability are terms of the log-linear model with scaled log-likelihood statistics (G^2/C). The significance of each term is determined from differences between adjacent models. N, nucleus; C, cytoplasm; R, reciprocal; S, sex. The interaction terms are all interactions with the segregation ratio.

square divided by its degrees of freedom. ANDERSON *et al.* (1985) show that this procedure is equivalent to correcting by an effective sample size, and it is analogous to the use of error variance in testing the significance of terms in analysis of variance. We obtained a heterogeneity χ^2 of 5473.14 with 2376 df, yielding a scaling factor of 2.304. This is rather larger than the scaling factor obtained by ANDERSON *et al.* (1985), and the reason is undoubtedly due to the inflated temporal heterogeneity we observed. Table 2 reports the significance of terms obtained from differences between adjacent log-linear models. Note that in agreement with the linear models, the nuclear × cytoplasmic interaction is highly significant and that sex plays only a minor role. The log-linear model indicates a significant contribution of a few more terms than the linear model, and this may be due to the nonconstancy of error variance. The temporal variation leads to a two-tiered hierarchy of clustering and may cause a departure from the Dirichlet-multinomial sampling distribution.

Fecundity analysis: The same model for analysis of variance can be applied to the fecundity data, and results are presented in Table 3. The nuclear x cytoplasm interaction is again significant, as is the nuclear effect. Since the fecundity of a mating pair is largely determined by the maternal type, it is not surprising to find a significant reciprocal cross effect in fecundity. Fecundity is very dependent on microenvironmental variation; therefore, nuclear × block and cytoplasm × block interactions were also significant.

Temporal heterogeneity of fecundities was even more pronounced than that of segregations, and the significance of this heterogeneity was explored as previously. The cumulative components of variance again revealed a trend in

TABLE 3

Analysis of variance of log-transformed fecundity data

Source	d.f.	MS	Variance	% variance
N	5	2.6612*	0.0131 ± 0.0099	4.98
C	5	0.9154	0.0016 ± 0.0036	0.59
R	1	2.7819*		
B	11	0.7288	0.0045 ± 0.0041	1.70
NC	25	0.6062***	0.0188 ± 0.0069	7.16
NR	5	0.2061	−0.0025 ± 0.0021	0
NB	55	0.3221***	0.0140 ± 0.0051	5.32
CR	5	0.6078	0.0038 ± 0.0047	1.45
CB	55	0.2389*	0.0071 ± 0.0039	2.69
RB	11	0.1513	− 0.0026 ± 0.0023	0
NCR	25	0.3247*	0.0124 ± 0.0074	4.70
NCB	275	0.1541	−0.0111 ± 0.0099	0
NRB	55	0.2374	0.0101 ± 0.0078	3.87
CRB	55	0.1842	0.0013 ± 0.0062	0.50
NCRB	275	0.1763	0.1763 ± 0.0212	67.03

Format follows that of Table 1.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

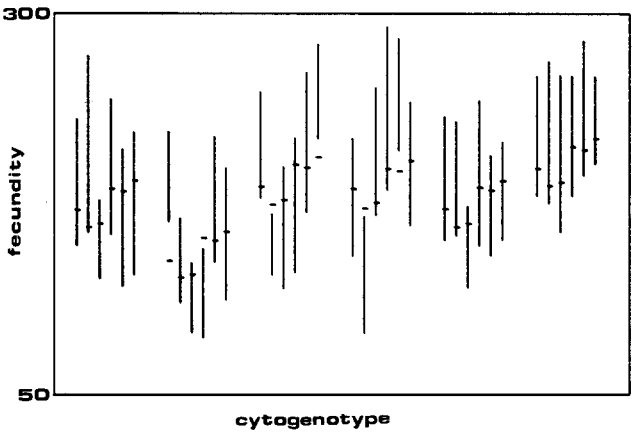

FIGURE 10.—Summary of fecundity data. Error bars represent means ±2 SE in fecundities (number of adult progeny per pair of duplicate vials) for each chromosome and cytoplasm combination, considering only reciprocals with wild-type female parents. The order of the bars is as in Figure 6.

error variance rather similar to the trend in segregation error variance. Other components of variance were not so consistent in their magnitude, but the nuclear effect and the nuclear × cytoplasm interaction tend to remain somewhat larger than the other components. Figure 10 provides a description of the fecundity data that is analogous to the presentation of Figure 6. Here, it is clear that the pattern of fecundities varies considerably over chromosomes and cytoplasms. In no case do flies bearing the *i*th chromosome in the *i*th cytoplasm show a fecundity that is significantly different from the value predicted by the additive model.

DISCUSSION

Although the study of cytoplasmic effects was motivated largely by the observation of extensive mtDNA and cpDNA variation, it is important to consider other factors that may be cytoplasmically transmitted. Four types of cytoplasmically transmitted material have been described in Drosophila. The first is a set of viruses, including the sigma virus, which is transmitted through the egg cytoplasm and confers sensitivity of adult flies to CO_2 anesthesia (L'HERITIER 1970). A nuclear gene designated *ref* renders sigma-bearing flies insensitive to CO_2, and this locus is polymorphic in natural populations, as is the presence of the sigma virus (FLEURIET 1976). The Drosophila C virus is an RNA picornavirus that is both cytoplasmically and horizontally transmitted in Drosophila (JOUSSET, BERGOIN and REVET 1977), and THOMAS-ORILLARD (1984) recently demonstrated quantitative phenotypic effects of infection by this virus. The second class of cytoplasmically transmitted variation in Drosophila is spiroplasmas (POULSEN and SAKAGUCHI 1981). These organisms survive within the cytoplasm of insect cells and have been implicated in causing reciprocal cross differences in interspecific hybrid sterility and maternally inherited "sex ratio" conditions in *D. nebulosa*, *D. paulistorum* and *D. willistoni* (MALOGOLOWKIN 1958). A third class of non-Mendelian phenomena in Drosophila is hybrid dysgenesis, which was first detected by the observation of reciprocal cross differences in fertility of hybrids of laboratory and wild-caught stocks (for review, BREGLIANO and KIDWELL 1982). The fourth class of cytoplasmically transmitted variation in Drosophila is mtDNA, and there has not yet been report of any phenotypic association with this variation.

Cytoplasmic transmission is generally inferred through analysis of reciprocal crosses, and it is essential to realize that there are a number of ways that reciprocal cross differences can be generated in Drosophila. These include (1) true cytoplasmic effects, (2) a maternal effect, mediated by nuclear genes but expressed in the egg cytoplasm (such as persistent maternal mRNA's), (3) meiotic drive in oogenesis, (4) meiotic drive in spermatogenesis, or differential sperm maturation, (5) sex-specific recombination and (6) hybrid dysgenesis. The experiment reported here was designed to control as many of these factors as possible. Although dysgenic crosses were avoided by extracting all stocks in the *P* cytotype, we cannot exclude the possibility of some *P* element transposition. We also cannot rule out the possibility of other analogous systems of nuclear × cytoplasmic interaction, although the absence of sterility indicates compatibility in the *I-R* system of dysgenesis as well. Sex-specific recombination can induce reciprocal cross differences whenever F_1 backcrosses are performed with inbred or isogenic lines and segregation is scored, because of the differences in viability conferred by recombinant and nonrecombinant gametes (CLARK and BUNDGAARD 1984). This possibility can be ruled out in the present design.

It is possible that the different second chromosomes extracted in this study confer different segregations due to meiotic drive, but these effects would be confounded with the nuclear effects rather than the cytoplasmic effect. A

nuclear × cytoplasmic interaction would be induced only if the meiotic drive were mediated by the cytoplasm, and the only case in which this is known to occur is hybrid dysgenesis. Maternal effects may also be involved in the observed segregation patterns, but here again, this phenomenon would be confounded with the nuclear effect rather than the cytoplasmic effect. The repeated backcrossing that was carried out before the segregation tests were performed further guarantees that the cytoplasmic differences are stably transmitted.

Previous investigators have analyzed factorial crossing designs for evidence of cytoplasmic effects using quadratic analysis (COCKERHAM and WEIR 1977; COCKERHAM and MUKAI 1978). In the latter case, significant maternal and paternal contributions to segregation variance were attributed to meiotic drive. After those experiments had been scored it was discovered that *P-M* dysgenic crosses were included, and one of the results of hybrid dysgenesis is altered segregation. In the present design, nuclear genomes were replaced in different cytoplasms and as a result the cytoplasmic effect is orthogonal to the reciprocal cross effect (*i.e.*, both reciprocal crosses were performed in all combinations of chromosomes and cytoplasms). Here, reciprocal crosses yielded the same segregation ratios, yet the cytoplasm of the stock modified adult phenotypic ratios.

The repeated backcrossing resulted in strains that had cytoplasms representative of the original wild cytoplasm only to the extent that the salient factors are strictly maternally inherited and that the transmission is temporally stable. There is evidence in cattle (HAUSWIRTH and LAIPIS 1982) and freshwater fishes (R. W. CHAPMAN, personal communication) that some individuals are not strictly homoplasmic, and in the latter case, paternal transmission was demonstrated. Cases of heteroplasmic individuals of Drosophila (SOLIGNAC, MONNEROT and MOUNOLOU 1983) and rats (BROWN and DESROSIERS 1983) all had variation in the nontranscribed D-loop region, and the variation was interpreted as being due to somatic mutation rather than paternal transmission.

The assumption of temporal stability of transmission seemed reasonable for mtDNA, but the extent of temporal variation seen in these experiments must be explained. In the classic experiments of MUKAI (1964), two lethals and three "deleterious" mutations were detected by the tenth generation. With an estimated polygenic mutation rate of 15% per second chromosome per generation there was certainly an induction of mutations in our lines, and the reextraction experiment verified the viability effects. Nuclear gene mutation remains the most likely cause of the increasing trend in the error variance but cannot account for the consistently observed nuclear × cytoplasmic interaction in segregation.

The flies that were used in the present experiment were derived from different populations; therefore, it is not possible to comment on the ability of the observed effects to maintain both nuclear and cytoplasmic variation within a panmictic population. In the case of cytoplasmic variation, theoretical results (GREGORIUS and ROSS 1984; CLARK 1984) suggest that sexual asymmetry may be important to the maintenance polymorphism, although no such asymmetry was apparent in the lines observed here. Despite the unexpected strength of

cytoplasmic effects reported here, we make no attempt at present to implicate mtDNA variation as the cause. Experiments are in progress to address these issues and to describe the extent to which variation in components of fitness are cytoplasmically transmitted within a panmictic population.

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