

Fitness and life-history traits of the two major mitochondrial DNA haplotypes of *Drosophila subobscura*

JS Christie¹, JA Castro¹, P Oliver¹, A Picornell¹, MM Ramon¹ and A Moya²

¹Laboratori de Genètica, Departament de Biologia, Facultat de Ciències, Edifici Guillem Colom, Universitat de les Illes Balears, Campus de la UIB, Palma de Mallorca, Balears 07122, Spain; ²Institut 'Cavanilles' de Biodiversitat i Biologia Evolutiva i Departament de Genètica, Universitat de València, Burjassot, València, Spain

Mitochondrial DNA restriction site analyses on natural populations of *Drosophila subobscura* have proved the existence of two common, coexisting haplotypes (I and II), as well as a set of less frequent ones derived from them. To explain this distribution, experiments to date point practically to all possible genetic mechanisms being involved in the changes of gene frequencies (cytonuclear coadaptation, direct natural selection on mtDNA and genetic drift). In an attempt to find differences that help to understand the dynamics of these haplotypes and to detect the effect of selection, we measured certain fitness components and

life-history traits (egg–larva and larva–adult viabilities and developmental times, longevity, resistance to desiccation and optimal density) of the two main haplotypes I and II when maintained in laboratory population cages. As a general trend, haplotype II showed a higher net fitness than haplotype I, which explains the superiority of haplotype II over haplotype I in experimental populations but not their coexistence in nature, where additional factors must be considered.

Heredity (2004) 93, 371–378. doi:10.1038/sj.hdy.6800513
Published online 7 July 2004

Keywords: fitness components; natural selection; cytonuclear interactions; mtDNA haplotypes; *Drosophila subobscura*

Introduction

It is a constant observation in the Old World that *Drosophila subobscura* shows widespread geographical homogeneity with a high prevalence of two main and coexisting mitochondrial DNA (mtDNA) haplotypes at the level of restriction site analyses (named I and II), as well as a set of less common haplotypes, derived from these two, which appear at much lower frequencies (Latorre *et al.*, 1992; García-Martínez *et al.*, 1998; Castro *et al.*, 1999; Oliver *et al.*, 2002). Studies on populations colonizing the New World have given similar results (Rozas *et al.*, 1990). The unique exception to date has been detected on the Canary Islands, where an endemic haplotype (named VIII) is predominant on some islands (Pinto *et al.*, 1997).

In recent years, we have studied the dynamics of these haplotypes, trying to answer the questions as to why the two main haplotypes are approximately equally frequent in nature and which evolutionary forces are implicated. Moya *et al.* (1993) studied the degree of differentiation of the two haplotypes at the nucleotide level. A total of 2377 bp from six mtDNA functional regions, representing 15% of the mtDNA genome, were sequenced per haplotype. Only three differences were found and they

proved to be silent changes at the protein level, one of which corresponded to the *Hae*III restriction site, located in the ND5 gene, which distinguishes the haplotypes. On the basis of these results, as well as others involving the geographical distribution, they considered haplotypes I and II to be phenotypically equivalent, although it is possible (and even probable) that further sequencing could reveal significant differences between the two mtDNAs in the remaining 85% (Moya *et al.*, 1993). In fact, when these haplotypes were put together at different frequencies in population cages, haplotype II always displaced haplotype I (García-Martínez *et al.*, 1998). Likewise, in another study, when haplotypes I and VIII were put together to compete in population cages at different densities and nuclear backgrounds, the mtDNA haplotype that reached fixation was the one placed within its own nuclear background, thus indicating the importance of nuclear–mtDNA coadaptation (Fos *et al.*, 1990). More recently, Castro *et al.* (1999) and Oliver *et al.* (2002) proved the existence of transient cytonuclear disequilibria, with nuclear allozyme loci and chromosomal arrangements. In summary, different studies to date have pointed practically to all the populational mechanisms that can change gene frequencies (cytonuclear coadaptation, direct natural selection on mtDNA and random genetic drift) as being forces potentially acting on the mtDNA haplotypes.

The present research falls within the series of studies that many groups have been carrying out in recent years, on the populational forces that are acting on the mtDNA. In the literature, studies concerning the population genetic dynamics of mtDNA have often, although not

Correspondence: JA Castro, Laboratori de Genètica, Departament de Biologia, Facultat de Ciències, Edifici Guillem Colom, Universitat de les Illes Balears, Campus de la UIB, Palma de Mallorca, Balears 07122, Spain.
E-mail: jose.castro@uib.es

Received 23 October 2003; accepted 1 April 2004; published online 7 July 2004

always, rejected neutral patterns, mainly in humans and their associated commensal taxa (flies and mice) (see, Gerber *et al*, 2001, for a recent revision). Similarly, studies on other species of *Drosophila* have also observed non-neutral behaviour of mtDNA variants (Hutter and Rand, 1995) and have provided evidence that maintenance of mtDNA variability could be mediated through cyto-nuclear interactions (Clark and Lyckegaard, 1988; MacRae and Anderson, 1988; but see Singh and Hale, 1990, for an alternative view). Rand *et al* (2001) recently showed that nuclear-cytoplasmic polymorphisms could also be maintained by interaction between X-chromosomes and cytoplasm.

In the present work, research was focused on fitness components and life-history traits as components of natural selection. These components have been extensively reported in the literature for several organisms. In *Drosophila*, the adaptation to novel environments involving high-density conditions has been studied (Mueller *et al*, 1993), as well as senescence (Rose, 1984), starvation (Rose *et al*, 1992), temperature (Huey *et al*, 1991), fecundity, longevity and developmental time (Matos *et al*, 2000), and population divergence (Kennington *et al*, 2001) among others.

Therefore, the purpose of this work was the detection of the differential action of natural selection on flies with these mtDNA haplotypes. The study was a continuation of the work we have been undertaking on the forces acting on the populational dynamics of the two haplotypes (I and II). Thus, we studied the egg-larva and larva-adult viabilities and developmental times, longevity and the resistance to desiccation. In addition, we also studied the optimal density (OD) (density at which the food medium yields the highest number of adult individuals) to measure the way in which the medium is exploited (Wallace, 1981; Castro *et al*, 1985). Other fitness components, such as mating pattern and female fertility, which are especially important in mtDNA dynamics due to maternal inheritance, were studied in flies from population cages (the same used in the present paper) and in a recently collected lines from a natural population (Castro *et al*, 2003).

Materials and methods

Haplotype populations

Two different populational cages were founded, one with haplotype I and another with haplotype II. The cage with haplotype I was created with 71 isofemale lines and the cage with haplotype II with 98. The isofemale lines were collected in a pine forest near Calvià (Majorca, Spain) in the spring of 1997, and the mtDNA haplotypes determined as indicated in Castro *et al* (1999). Each isoline contributed approximately five males and five females. They were maintained in the laboratory at 19°C, 70% relative humidity, a 1:1 day-night cycle and with 12 jars containing corn-meal food. The mean number of individuals was approximately 1500 per generation. From these cages, two samples, one with 18 haplotype I isofemales and another with 21 haplotype II isofemales, were obtained in order to establish separate populations for the experiments. When these samples were taken, the cages had passed nine discrete generations. The mtDNA haplotypes of these isofemales were checked through the

offspring by a simple mtDNA analysis (Castro *et al*, 1999). The use of 18 and of 21 isofemale lines, respectively, should be enough to capture 95% of the variability in nuclear alleles that is present in the cages (Hedrick, 2000, p. 240). These two new populations were maintained by serial transfer in five 500 ml bottles, during the time that the experiments were being performed.

Presence of Wolbachia

To exclude an incompatibility system in *D. subobscura*, promoted by the presence of *Wolbachia*, a PCR assay using 16S rDNA *Wolbachia*-specific primers was carried out following the methodology of García-Martínez *et al* (1998). Six lines were used at random (three of haplotype I and three of haplotype II) from the Calvià population before creating the cages. Since the results were negative, the presence of *Wolbachia* in the population was excluded. We only used a few lines of *D. subobscura* because this determination was the second one made on the island of Majorca. The first one was in a different population (Esporles) a few years ago and was also negative (García-Martínez *et al*, 1998). Moreover, we have not detected any kind of cytoplasmic incompatibility in the population, such as embryonic death or sex ratio distortion in crosses with different isofemale lines.

Experiments

Egg viability and developmental time: Four groups of approximately 15 adult couples per haplotype were transferred for 24 h from the 500 ml maintaining bottles to 250 ml bottles with fresh food in order to provide sufficient adult offspring reared without competition. At 2 or 3 days after eclosion, offsprings (males and females together) were transferred to new 250 ml bottles with fresh food, where the flies could mate, feed and lay eggs freely for 1 week. These flies with 9–10 days of age were used for egg laying. They were transferred for 1 h to 'oviposition vials', which contained a watch glass with agar, water, acetic acid, ethyl alcohol and a few milligrams of live yeast.

The eggs were laid on three watch glasses per haplotype and were immediately ordered into columns. Eggs were checked at 0, 14, 23, 35 h, and then at intervals of 30 min until 48 h, before being checked the next day at 62 h. Hatching was deduced from the number of empty eggs found. Four replicates were carried out on 4 consecutive days and the number of eggs used in each was 161/160, 125/130, 125/147 and 74/69, for haplotypes I and II, respectively. This procedure allowed the variations in developmental time and egg viability of each haplotype to be followed.

Larva-adult viability and developmental time without competition: Once having obtained eggs as in the previous experiment (except that egg laying lasted 2 h instead of one), they were kept in Petri dishes for 48 h at 19°C until the larvae hatched. Then 50 larvae of the same age were seeded in 10 × 3 cm tubes with 10 ml of fresh food. Larvae were picked up one by one directly from the watch glasses with a lancet under a microscope. The adults that emerged were counted daily, and a total of 13 replicates were carried out for each haplotype.

Viability was expressed as:

$$V = \frac{n}{N}$$

where N is the input number of larvae and n is the output number of adults emerging from these N larvae.

Developmental time (DT) was measured in days by the formula:

$$DT = \frac{\sum N_i d_i}{\sum N_i}$$

where N_i is the number of flies emerging d_i days after the larvae were placed on the medium.

Longevity: The flies tested for longevity were the offspring taken straight from the isofemale line population founder tubes, as they hatched from their pupae. Approximately 30 individuals of each haplotype and sex were maintained individually in 10×3 cm tubes with 10 ml of food medium containing active yeast on the surface. Flies were checked daily. Vials were changed every fortnight, before dehydration of food was evident. All the flies that escaped, were damaged or died during the experiment were included in the analyses.

Resistance to desiccation: In all, 30 flies of 8 days of age per haplotype and sex were put individually in empty 10×1.1 cm tubes, and they were checked hourly from the 23rd h until death. Flies for experiments were obtained as above.

Optimal density: Larvae were obtained with the same methodology indicated in the second experiment. Larvae were seeded in 7.5×1 cm tubes with 0.75 ml of food, in increasing densities of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 larvae. Adults emerging at each level were counted daily, and a total of four replicates were carried out for each haplotype.

Statistics

Statistical analyses were performed by means of the SPSS v 11.5 and the BIOM (Sokal and Rohlf, 1995) packages. Viabilities were subjected to the arcsine-square root transformation for statistical analyses, but the results with transformed data were qualitatively indistinguishable from analyses with untransformed data, so only the latter are presented. The longevity and the resistance to desiccation were analysed by means of the survival analysis procedure (included in the SPSS package). The method was that of Kaplan–Meier survival curves. The analysis gives, among others parameters, the cumulative survival with time and the mean with standard error (SE) for each curve. The comparison of the several curves was made with the log-rank test, so called because it can be shown to be related to a test that uses the logarithms of the ranks of the data. We made comparisons of the four curves globally and by pairs. To diminish any statistical artefacts in the significance that could arise when multiple comparisons are made, a sequential Bonferroni test (Rice, 1989) was applied to correct the probabilities when many tests are carried out simultaneously.

E -statistic (Moya *et al*, 1986) is a fitness parameter that combines data of viabilities and developmental times, and follows the optimality principle that the largest

number of adults emerged in the shortest possible time gives the best fitness. The characteristic of this parameter is that it simultaneously maximizes viability, V , and the reciprocal of the development time, DT , as follows:

$$E_{\max} = \max\left(V \frac{1}{DT}\right)$$

The function adopted for E is

$$E = \sum_{i=0}^k \frac{V_i}{T_i}$$

where $V_i = s_i/N$ and $T_i = t_i/t_{\text{optimal}}$; s_i is the number of individuals emerged at t_i time; N is the input number; and t_{optimal} is a conventional minimum developmental time (in the egg–larva test it was considered as 40 h, and in the larva–adult test as 20 days).

Hence, for computational purposes we can use

$$E = \frac{t_{\text{optimal}}}{N} \sum_{i=0}^k \frac{s_i}{t_i}$$

It is worthy of remark that E -statistic is only a combination of viabilities and developmental times; thus, it does not include other fitness components such as population growth rates or fecundities.

Results

No differences in the egg-to-larva viability, developmental time or the E -statistic were statistically significant, although the trend was the same as found by Matos and Rocha Pité (1989) for young female flies, where percentages for eclosion showed very high values, close to or attaining 100%. Flies with haplotype II had higher viability and developed faster than haplotype I (Table 1). The same table shows the larva-to-adult viabilities, developmental times and E -statistic, always favouring haplotype II, although statistically different were only found in the DT and E . However, in both experiments, the developmental times were longer than those of previous studies (Rocha Pité, 1982) where this species developed faster even at lower temperatures. In both strains, the viabilities were low and in the same range as those found in the literature (Budnik *et al*, 1991).

The survival curves for the adult males and females of both haplotypes are shown in Figure 1. As can be observed in Table 2, the females of both haplotypes have similar mean longevity and lived longer than males. The survival analyses showed a great similarity in the dynamics of both females, but not in males, different between them and in relation to the females, as indicated by the log-rank tests (Table 2). Longevity results were similar to those of Maynard Smith (1956) in that many female flies were alive 100 days after eclosion, but they were dissimilar to Clarke and Maynard Smith (1955), whose flies all lived less than 96 days.

Haplotype II was more resistant to desiccation than haplotype I in both sexes, as can be seen in Figure 2. Within the haplotypes, females were more resistant than their corresponding males. These results are in accordance with those obtained by Levine (1986) for *D. pseudoobscura* and *D. melanogaster*. All comparisons were significant except between haplotype II males and both female haplotypes (see Table 2).

Table 1 Means with SEs for the egg–larva and larva–adult V , DT and E -statistic for haplotypes I and II

	Haplotypes		t-Test	df
	I	II		
Egg–larva				
V	0.968 ± 0.014	0.988 ± 0.006	1.32 (NS)	6
DT (hours)	42.49 ± 0.18	42.15 ± 0.40	0.77 (NS)	6
E	0.914 ± 0.016	0.943 ± 0.012	1.42 (NS)	6
Larva–adult				
V	0.632 ± 0.024	0.689 ± 0.023	1.73 (NS)	24
DT (days)	24.75 ± 0.20	23.21 ± 0.08	6.99***	24
E	0.512 ± 0.017	0.595 ± 0.019	3.19**	24

df: degrees of freedom.

NS: not significant.

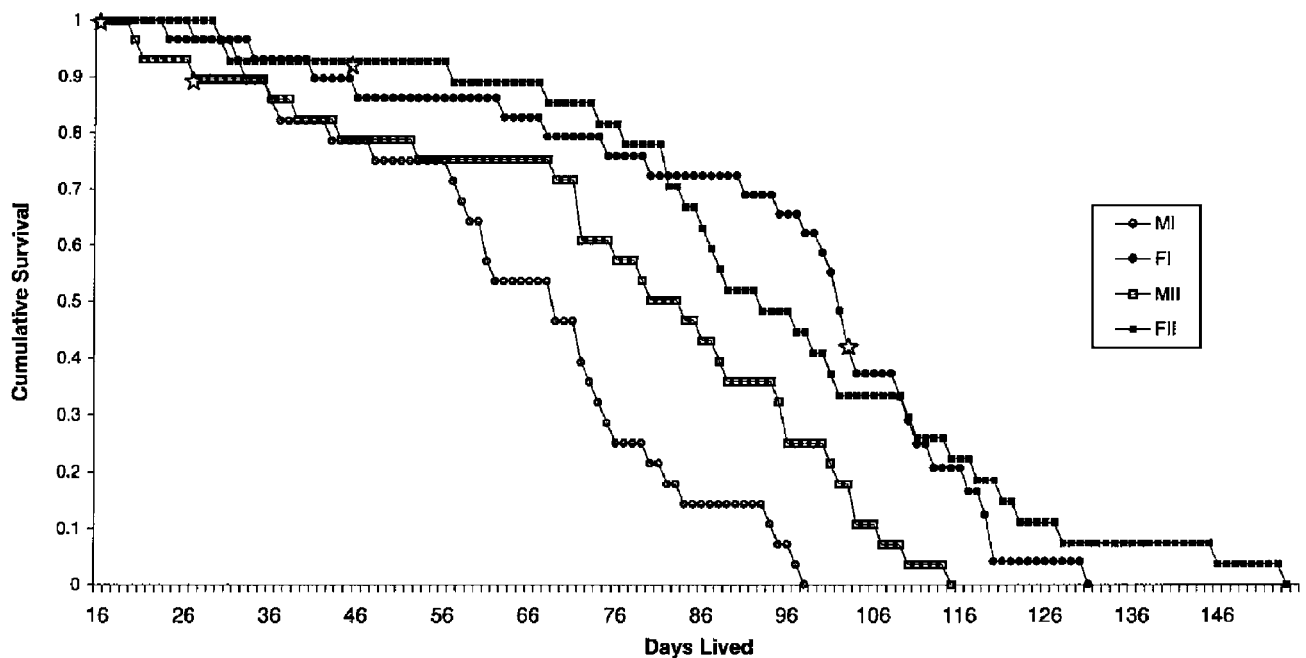
** $P < 0.01$.*** $P < 0.001$.**Figure 1** Survival curves for longevity (in days) for haplotypes I and II (MI and MII: males; FI and FII: females). Censored individuals are indicated by stars.

Table 3 shows the V 's, DTs and E -statistic with SEs for each density and haplotype from the OD test. As a general trend, haplotype II had a higher V but also a higher DT. E -statistic showed globally a higher value for haplotype II. In this experiment, only trends were detected in the results, because, overall, there were no statistically significant differences in the densities between the two haplotypes with respect to the three parameters (haplotype II in V and E were significantly higher than haplotype I in only the 10 and 60 density bands). It is possible that more replicates in this experiment would confirm these tendencies. Nevertheless, these results show that the differences between the two haplotypes were very small.

As expected, viability and developmental time were density-dependent parameters, with the first decreasing and the second increasing as the density increased. In the literature, it is reported that the best statistical fit of

these parameters is not always linear and it is common to fit the data to a second- or third-degree polynomial regression (see, for example, Castro *et al*, 1985 and references inside for a detailed explanation of the method). Table 4 shows the best fit of the V 's, DTs and E by density that corresponded to second-degree polynomial regressions based on the analysis of variance for both haplotypes. From the polynomial regression, optimal densities of 32 individuals for haplotypes I and II were obtained in viability, but haplotype II showed a slightly better use of the medium by having a higher number of survivors at this OD (11.7 *vs* 9.6). The results with E were similar (Table 4 and Figure 3). The curves in Figure 3 resulted from the polynomial regression for V and E multiplied by N . Only the first part of the curves (up to density 50) that include the OD values are represented, and the original survival points are also indicated. This methodology coincides with similar

experiments on *D. melanogaster*. The crowding conditions caused by progressive density provoke an increasing exploitation of the medium to a maximum level (the OD). From this point onwards, the limitation of resources and the increasing concentration of nitrogen catabolites from larval excretion cause a self-poisoning of the larvae, which explain, at least in part, the dynamics of the process, mainly at high densities (Castro *et al*, 1985; Moya and Botella, 1985; Moya and Castro, 1986; Botella *et al*, 1988).

Table 2 Means with SEs for longevity (in days) and desiccation resistance (in h) for sexes in haplotypes I and II. The log-rank tests based on the survival analyses are indicated (the global analysis and by pairs)

	Longevity	Desiccation resistance
MI	65.14 ± 3.88	35.57 ± 0.95
MII	76.54 ± 5.25	42.20 ± 2.12
FI	93.48 ± 5.27	39.55 ± 1.07
FII	94.74 ± 5.54	46.27 ± 2.05
Global	39.20*** (3)	28.44*** (3)
MI–MII	8.00* (1)	10.46** (1)
FI–FII	0.06 (NS) (1)	9.16** (1)
MI–FI	27.54*** (1)	6.32* (1)
MII–FII	6.59* (1)	1.38 (NS) (1)
MI–FII	21.86*** (1)	22.36*** (1)
MII–FI	9.38** (1)	3.38 (NS) (1)

MI and FI: males and females for haplotype I.
 MII and FII: males and females for haplotype II.
 NS: not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

The probabilities were corrected by the sequential Bonferroni test. Degrees of freedom are given within parentheses.

Discussion

We have tested the differences in some fitness and life-history components for the two main RFLP haplotypes, I and II, in an attempt to find differences that would help us to understand their populational dynamics. We have studied those characteristics commonly used in the literature (Hedrick, 2000), although we are conscious that the prediction of the outcome of natural selection based on separate fitness components could be risky (Prout, 1971a, b). As a general trend, it was observed that haplotype II was more efficient than haplotype I.

Egg viabilities, egg–larva developmental times and *E*-statistic gave no significant differences. The larva–adult developmental time was significantly shorter for haplotype II and the global *E*-statistic was higher. These results indicate an advantage over haplotype I because in population cages, with discrete generations, we can follow the optimality principle that the largest number of adults emerging in the shortest possible time gives the best fitness. In these laboratory conditions, flies that emerge earlier can mate and lay eggs earlier and, as a consequence, their larvae take the best place on the medium and so feed better. However, this explanation cannot be fully applied to natural conditions, because trade-offs may exist in such a way that slower could be better under certain conditions due to the complexity existing in nature. In relation to *E*-statistic, we think that it is a good approximation to the global fitness of viability and developmental time, although we are aware that the developmental time could have an exponential impact on reproduction, whereas the other traits (not just viability) may only be multiplicative.

For longevity, haplotype II showed a higher longevity trend in both sexes, although significant differences within sexes were found only in males. This advantage is relevant in females because the mtDNA has a maternal transmission, whereas it is not relevant in males because

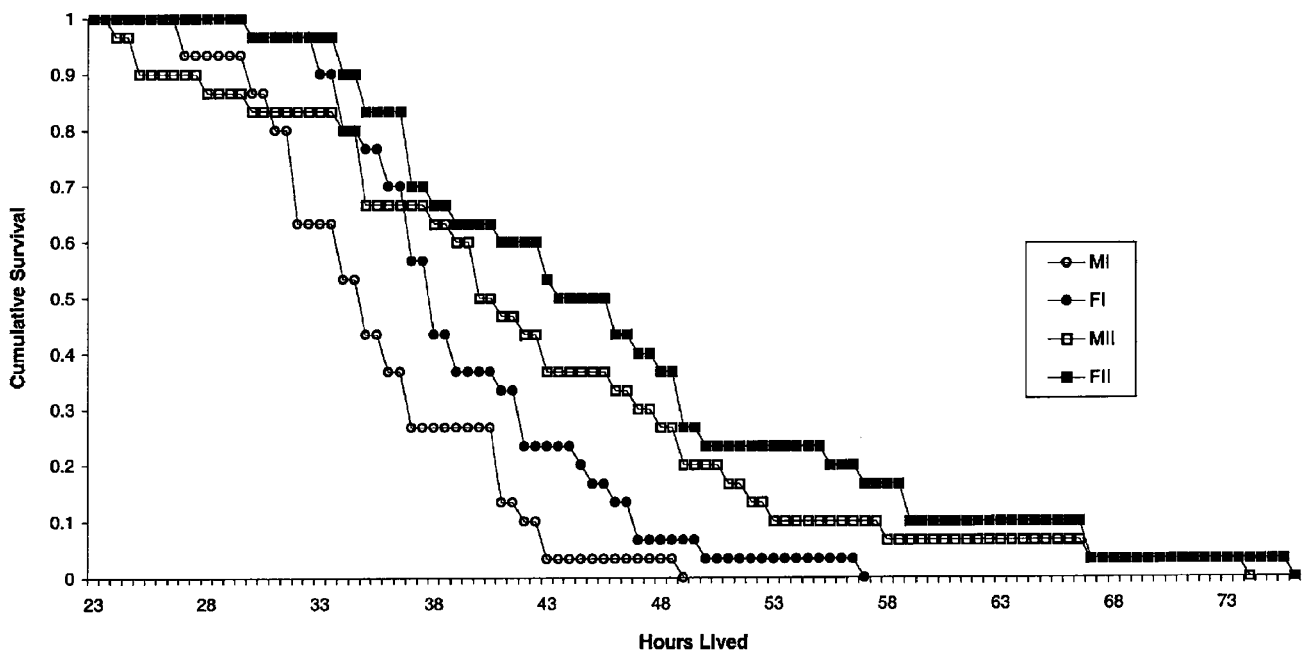


Figure 2 Survival curves for the desiccation resistance (in hours) for males and females of haplotypes I and II.

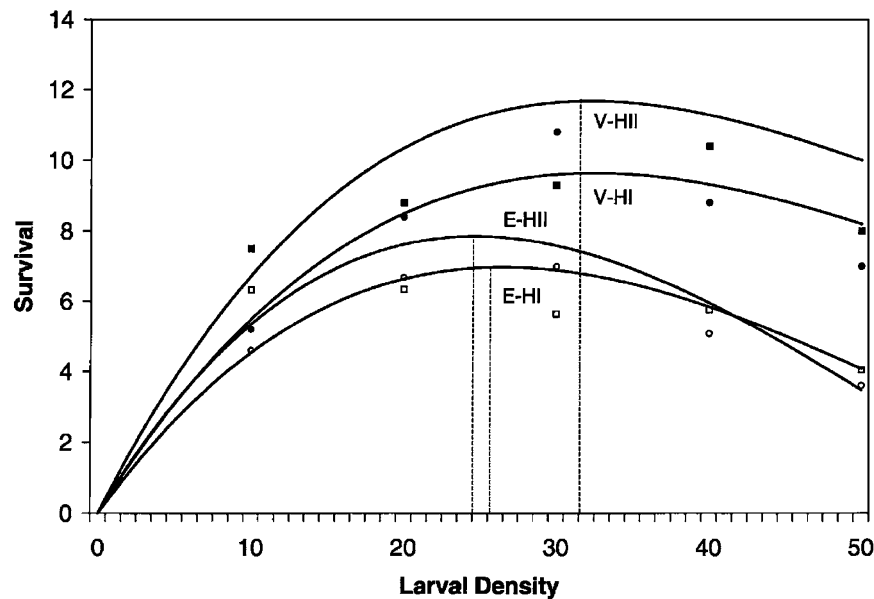
Table 3 Mean V 's, DTs (in days) and E -statistic with SEs for each density and haplotype

Larval density	V		DT		E	
	I	II	I	II	I	II
10	0.52 ± 0.07	0.75 ± 0.03	22.72 ± 0.25	23.81 ± 0.43	0.461 ± 0.062	0.632 ± 0.014
20	0.42 ± 0.04	0.44 ± 0.02	25.46 ± 0.79	28.02 ± 0.99	0.334 ± 0.027	0.317 ± 0.029
30	0.36 ± 0.07	0.31 ± 0.04	30.93 ± 0.83	34.27 ± 1.17	0.233 ± 0.045	0.188 ± 0.027
40	0.22 ± 0.05	0.26 ± 0.04	36.41 ± 0.86	36.53 ± 1.04	0.127 ± 0.029	0.144 ± 0.026
50	0.14 ± 0.02	0.16 ± 0.03	43.00 ± 2.29	42.84 ± 1.54	0.072 ± 0.014	0.081 ± 0.016
60	0.06 ± 0.02	0.16 ± 0.02	45.36 ± 2.85	44.75 ± 1.94	0.027 ± 0.008	0.078 ± 0.009
70	0.07 ± 0.01	0.11 ± 0.02	47.08 ± 2.04	45.92 ± 3.72	0.033 ± 0.006	0.050 ± 0.007
80	0.05 ± 0.01	0.06 ± 0.01	47.87 ± 3.29	53.01 ± 1.35	0.023 ± 0.008	0.024 ± 0.005
90	0.06 ± 0.02	0.08 ± 0.01	45.46 ± 4.24	50.61 ± 1.26	0.026 ± 0.010	0.033 ± 0.006
100	0.06 ± 0.02	0.07 ± 0.01	51.16 ± 3.08	54.20 ± 1.11	0.026 ± 0.012	0.029 ± 0.002

Table 4 Parameter values for the polynomial regression and OD (with the corresponding survival within parentheses) for each of the three fitness components in the two haplotypes

Fitness Components	Haplotype I			Haplotype II		
	Parameter values	R ²	OD (S)	Parameter values	R ²	OD (S)
V	$a = 0.689$ $b_1 = -1.5E-2$ $b_2 = 9E-5$	0.984	32 (9.6)	$a = 0.850$ $b_1 = -1.90E-2$ $b_2 = 1.20E-4$	0.951	32 (11.7)
DT	$a = 14.25$ $b_1 = 0.719$ $b_2 = -3.70E-3$	0.966		$a = 17.97$ $b_1 = 0.583$ $b_2 = -2.25E-3$	0.983	
E	$a = 0.594$ $b_1 = -0.015$ $b_2 = 9.5E-5$	0.990	26 (7.0)	$a = 0.712$ $b_1 = -0.019$ $b_2 = 1.23E-4$	0.921	25 (8.0)

R² measures the proportion of the experimental variance explained by the regression.

**Figure 3** Viability (V) and E -statistic (E) as a function of the input densities for the two haplotypes I and II. The predicted function is shown. The vertical dash lines indicate the optimal input density. The original survival points are also represented (VHI: ●; VHII: ■; EHI: ○; EHII: □).

they do not transfer it. Nevertheless, males contribute with nuclear genes, which generates the nuclear variability necessary in cytonuclear relationships. In this sense, as indicated above, we also consider the mating

pattern relevant, and this is an experiment that we carried out (Castro *et al*, 2003); in this experiment, the mating pattern indicated an assortative mating in population cages, where couples of the same haplotype

mated more often. Nevertheless, in wild populations, random mating was the rule.

In resistance to desiccation, both males and females of haplotype II are superior to haplotype I. Ecologically, this would mean a higher fitness in haplotype II flies during the driest periods of the year. This is supported by González *et al* (1994) whose autumn samples (flies and descendants of flies just after the dry season) showed larger frequency differences between the two main haplotypes in favour of haplotype II compared to their spring samples. The cause of a difference between haplotypes and not just sexes could be explained by differences in certain proteins that would result in a different functionality with conditions of rising cell osmolarity during the dehydration of the fly. Interestingly, Kennington *et al* (2001) showed, by means of this trait, the importance of the maternal contribution to population differentiation in *Drosophila melanogaster*.

In the OD experiment, the general trend was for haplotype II to make the best use of the medium, since the survival of haplotype II at OD (which has some similarities with carrying capacity in population dynamics studies) was higher than that of haplotype I. This means that haplotype II has a better fitness than haplotype I so that it can profit from the laboratory medium, and it is possible that this advantage persists in nature when seasonal conditions are extreme. However, the interpolation from monocultures to dicultures must be treated with caution, because the response pattern of both haplotypes competing together is not fully predictable from monocultures due to intergenotypic competition (Castro *et al*, 1985).

The differences in fitness component values found in the present work raise certain questions. (1) What are these fitness components measuring? We think that selection is acting differentially on the two haplotypes, either directly on the mtDNA or by some kind of selective coadaptation between the nuclear and the mtDNA genomes (epistatic selection and/or hitchhiking on mtDNA haplotypes). At a nuclear level, we can reasonably assume that both haplotypes had the same nuclear variability when the populational cages were founded, because of the high number of isofemale lines used for each haplotype. At the mtDNA level, 15% of the sequence of the mtDNA molecule showed three silent changes, including that of *HaeIII*. It is expected (and is probably true) that more differences could be found (not necessarily silent) in the remaining 85%. With our experimental design, we cannot distinguish clearly between factors that are exclusively from mtDNA for nuclear–mtDNA interactions, although we can say that the mtDNA is always implicated. Previous data (Castro *et al*, 1999; Oliver *et al*, 2002) showed the presence of cytonuclear disequilibria, permitting the possibility of some kind of epistatic selection. Despite the transience of the cytonuclear interactions (Babcock and Asmussen, 1996), in the cages and after nine generations, it is possible that some could be formed (differently in both cages) in response to laboratory environmental homogeneity, and therefore persist over time (or reappear recurrently). The consequence would be a net superiority of haplotype II over haplotype I, because the former could have developed a better functional coadaptation than the latter, as also indicated by Fos *et al* (1990). This would be mainly in the nuclear genes related to the more

relevant fitness components (resistance to desiccation, longevity and optimum density). (2) Can these differences be extrapolated to nature? The life in a population cage is very different from nature (no microhabitats, no differences in food, the same temperature and humidity, the nonexistence of early morning dew, a closed environment with competition for food and space, etc.), so a direct extrapolation to nature is not possible. In fact, Castro *et al* (2003) found, in relation to female fertility, that the adaptation to laboratory conditions gave a more global efficiency in the production of the offspring. In this situation, we can only say that under laboratory conditions, flies with haplotype II have a higher fitness than flies with haplotype I, and, thus, haplotype II would displace haplotype I in competition experiments. This was exactly what García-Martínez *et al* (1998) found in population cages. Therefore, our experiments could explain, at least partially, the displacement of haplotype I by haplotype II in those experiments. In nature, the situation is more complicated than in laboratory cages, and the superiority of one haplotype must be counterbalanced by the other in different situations. The demographic structural characteristics in *D. subobscura* have shown significant differences between seasons (Matos and Rocha Pité, 1989). Since patterns differ remarkably throughout the year, a seasonal analysis could be important to characterize the demographic parameters of each haplotype, as one haplotype is favoured momentarily to the detriment to the other. Therefore, flies with haplotype I must have some advantage over flies with haplotype II in order to explain the proportions found in nature, developing cytonuclear interactions, even transient, that could have selective importance in a changing environment.

Finally, we have to consider the sex-by-genotype interactions because they could, in part, maintain the variation in the populations. In this way, Rand *et al* (2001) developed a model for *D. melanogaster* in which nuclear-cytoplasmic polymorphism could be maintained by selection in X chromosome–cytoplasm interactions. They showed experimentally significant sex-by-genotype interactions for mtDNA haplotype, cytoplasm (the factors inherited through the female cytoplasm in *Drosophila* with different mtDNA, such as *Wolbachia*, σ and C virus and maternally loaded mRNA) and X chromosomes. Moreover, these interactions were sexually antagonistic (the good cytoplasm in females was bad in males). This model reveals the complexity of the interactions that we have to take into account.

Acknowledgements

This work has been supported by Grant PB96-0793 and BOS2000-1000 from the Dirección General de Enseñanza Superior (Ministerio de Educación y Cultura, Spain).

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