Is There Selection on RFLP Differences in Mitochondrial DNA?

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Manuscript received November 15, 1989
Accepted for publication March 30, 1990

ABSTRACT

Experimental populations of *Drosophila simulans* were established for the purpose of detecting the presence or absence of selection on a restriction fragment length polymorphism in mitochondrial DNA (mtDNA). It was then discovered that the founding strains differed with respect to the Rickettsia-mediated incompatibility system in this species, which is maternally transmitted together with the mtDNA differences. A population model was constructed using the known fitness effects of the incompatibility system, with the result that the population trajectories can be completely explained by the effects of the microorganism with no need to invoke selection on mtDNA. The strong conclusion is that in this case we can rule out the strong selection proposed by MacRae and Anderson to explain the "dramatic mtDNA changes" in their *Drosophila pseudoobscura* populations. The population theory used for the experiments is discussed in the context of natural populations. Estimated parameters include the possibility that with two populations, one with the organism and one without it, there may be no bias as to which will invade the other, which in turn suggests no global tendency for the infection to spread or decline.

MACRAE and ANDERSON (1988) have recently reported evidence for selection on restriction fragment length polymorphism (RFLP) differences in mitochondrial DNA (mtDNA). For this purpose they exploited the mtDNA differences between *Drosophila pseudoobscura pseudoobscura* and *Drosophila pseudoobscura bogotana*. In experimental populations descended from a cross between these subspecies the mtDNA differences were apparently subject to selection. In reciprocal crosses these two subspecies exhibit partial incompatibility due to some form of maternal effect (PRAKASH 1972; DOBZHANSKY 1974; ORR 1988).

We wish to report the results of a similar experiment with *Drosophila simulans*. Experimental populations were initiated with the objective of investigating the presence or absence of selection on mtDNA differences. Subsequent to the start of the experiments HOFFMAN, TURELLI and SIMMONS (1986) and HOFFMAN and TURELLI (1988) described an incompatibility system in this species mediated by a maternally transmitted Rickettsia. We than determined that our founding strains, upon reciprocal crossing, exhibited this incompatibility difference, thus creating a similarity between our experiment and those of MACRAE and ANDERSON.

We simply wish to report that all of the mtDNA frequency changes we observed can be adequately explained by the incompatibility system and that there is no need to invoke any selection on the mtDNA.

MATERIALS AND METHODS

Two strains of *Drosophila simulans* were used. These were collected in 1985, one in Padova (Veneto), strain P, and the other in Cortona (Toscana), strain C. There are mtDNA restriction fragment differences between the strains when their mtDNA is digested with endonuclease *Hpa*II. Two sets of population cages were established: three replicate cages had the initial frequency of the C mtDNA type of 0.80, the "high lines," and three had an initial frequency of the C type of 0.20, the "low lines." The cages were made of plexiglass and of dimensions 12 × 24 × 28 cm with 10 openings for 4 × 11-cm bottles with medium made with sucrose, agar, brewer's yeast and nipagin mold inhibitor. A continuous population was maintained by exchanging 5 bottles with fresh medium for the set of 5 older bottles every 20 days. The populations were kept at 22°. In order to randomize the mtDNA differences with any nuclear genetic differences, reciprocal crosses were made using 100 females from each strain. The progeny lines from these two crosses were then expanded and the populations were founded with 700 nonvirgin females of the appropriate mix for the high and low lines.

Samples of 40 females were then taken on days 18, 80, 130, 250 and 400. From each female an enriched fraction of mtDNA was extracted by the method of COEN, THODAY and DOVER (1982). This DNA was then digested with *Hpa*II and the digests subjected to electrophoresis using 8% agarose gels in TBE buffer (pH 8.3) and stained with ethidium bromide.

For the incompatibility tests between the two strains single females were placed in individual vials together with two males and allowed to mate and lay eggs for 2 days. All resulting progeny were counted. One-day-old males were used for one set of crosses and 4-day-old males used for the other set.

RESULTS

The C and P strains were tested by HOFFMANN and TURELLI and one of us (T.P.) against the standard "R"

TABLE 1

Incompatibility tests

Cross[a] ♀	♂	One-day-old males: Progeny per vial[b]	N[c]	SE[d]	Four-day-old males: Progeny per vial	N	SE
1) P × P		69.38	8	5.01	63.29	17	4.03
2) C × C		60.92	9	5.08	58.33	12	2.84
3) P × C		5.45	10	1.88	21.28	18	2.79
4) C × P		63.20	13	6.24	60.53	17	2.30

Results of Wilcoxson tests

Comparison	One-day-old males	Four-day-old males
1 *vs.* 2	NS	NS
1 *vs.* 3	***	***
1 *vs.* 4	NS	NS
2 *vs.* 3	***	***
2 *vs.* 4	NS	NS
3 *vs.* 4	***	***

[a] P and C ≡ mtDNA types.
[b] Mean number of progeny per vial.
[c] N ≡ number of vials.
[d] SE ≡ standard error.
*** ≡ $P < 0.001$.

and "W" strains established by HOFFMANN, TURELLI and SIMMONS with the result C ≡ R and P ≡ W. This result is reported in HOFFMANN and TURELLI where our C strain is identified as "Tuscany" in their Table 2 (on page 437) and their statement "W populations also occur in Europe" on the same page refers to our "P" strain.

HOFFMANN, TURELLI and SIMMONS showed that "R" is cured by tetracycline, and electron microscope studies by BINNINGTON and HOFFMANN (1989) demonstrated the presence of the Rickettsia *Wolbachia*, sp. in R but not W strains. Electron microscopy by LOUIS and NIGRO (1989) shows that the C strain has the Wolbachia but not the P strain. Table 1 shows the results of crosses to test for unidirectional incompatibility between the original C and P strains. Wilcoxon significances tests are given. The semisterility of the P♀ × C♂ cross, with less sterility of the older males, is characteristic of the R,W pattern.

Figure 1 shows the results of sampling the three replicate high lines and three low lines. Tests for heterogeneity among the three replicates gave only one significant G test with $0.01 < P < 0.025$ so the replicates were combined as shown in Figure 2 together with a predicted trajectory to be discussed presently. On day 400 at the end of the experiment 100 isofemale lines were established. Females from these were crossed to males from the original C stock, and also the mtDNA type was determined for each line. Thirty-two lines had C mtDNA and the females were fully fertile with C stock males. Of the 68 lines with P mtDNA, 66 were semisterile with C stock males, but two lines show the high fertility characteristic of a compatible cross. These had apparently acquired the microorganism.

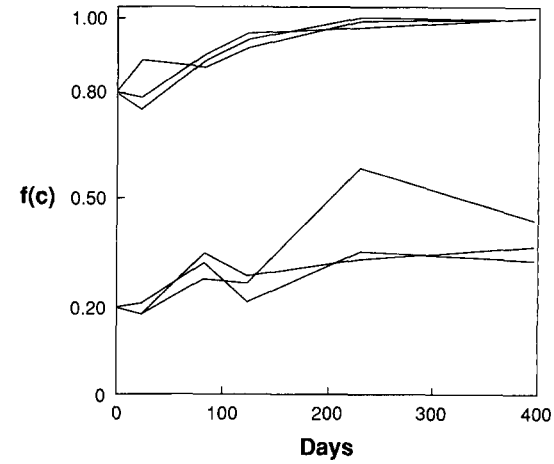

FIGURE 1.—Results of sampling populations. f(C) ≡ the relative frequency of mtDNA type C.

ANALYSIS AND CONCLUSIONS

Ideally the C mtDNA type should serve to monitor the incidence of the microorganism because both are transmitted matroclinously. However, HOFFMANN and TURELLI have evidence for a low frequency of transmission through males. Thus in a P♀ × C♂ cross, among the small number of resulting progeny approximately 1.4% acquire the infection. These individuals would be of the kind observed at the end of the experiment and are here denoted P_1, to indicate P mtDNA infected with microorganism. HOFFMANN and TURELLI also tested for spontaneous curing and found none. This result is also supported in our case by the finding that at the end of the experiment where females from all of the C mtDNA isofemale lines were compatible with C stock males. This means that in our population initiated with C infected and P not infected only a third type should appear, namely P_1, although our monitoring could not distinguish between P and P_i. The appearance of P_1 we here call "leakage."

Table 2 sets forth a discrete generation model for the three types with $f(C) \equiv p_1$, $f(P) \equiv p_2$ and $f(P_1) \equiv p_3$. Recurrence equations (2) refer to an age structured version to be discussed presently. There are three parameters: U the fitness effects of the microorganism on its carriers as compared to those not carrying it; V, the fertility of the incompatible cross and L, the leakage rate or the conditional frequency of infected individuals among the V individuals from the incompatible cross. With low leakage, L, and $f(P_1)$ small as at the start of the experiment when $f(P_1) = 0$ there is an unstable equilibrium at $f(C) = \hat{p}_1 = (1 - U)/1 - V)$. The objective here is to obtain parameter values from independent experiments and use these in the model to compare the predicted dynamics with those ob-

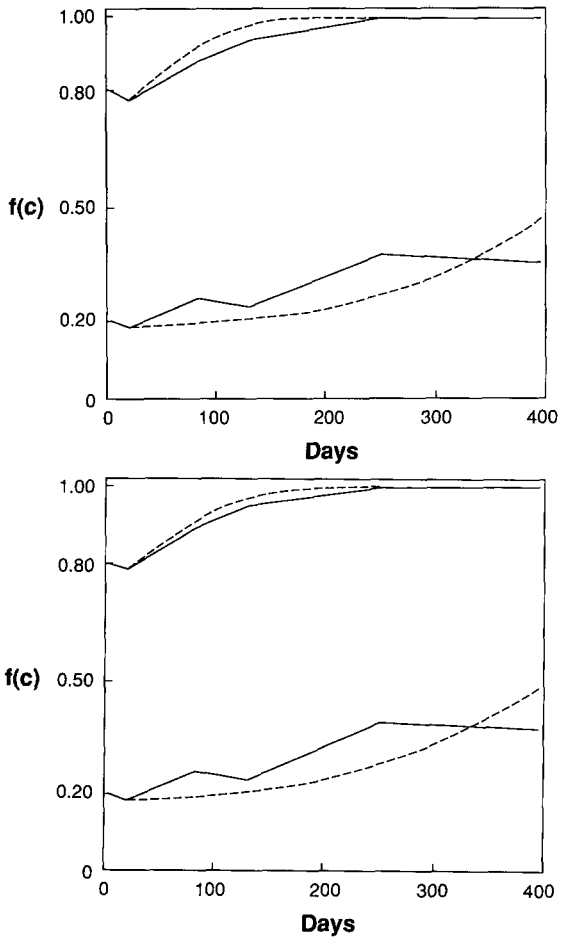

FIGURE 2.—(A) Solid lines are means of three replicas shown in Figure 1. Dashed lines are the trajectories given by the model. Parameters given in the text. (B) Solid lines as in (A). Dashed lines are the model trajectories assuming an "8-day week" with parameters given in the text.

served for $f(C)$ *vs.* $f(P) + f(P_1)$ in the experimental population.

HOFFMANN and TURELLI determined that there is a significant fitness effect of the microorganism amounting to $U = 0.781$ (SE = 0.046). The results shown in Table 1, although not statistically significant, are consistent with the HOFFMANN and TURELLI values. The ratio of the mean productivity of C × C and C♀ × P♂ crosses to that of P × P gives $U = 0.894$ for 1-day-old males and $U = 0.939$ for 4-day-old males. This "productivity" fitness does not resolve the fertility and viability components. With strictly matroclinous transmission with no intervening Mendelism these two components collapse so that it makes no difference whether the net fitness is formally treated as fertility as in Table 2 or viability. The only place in this system where the two components should be resolved is in the P♀ × ♂C cross where L of the offspring are infected. However, this is of such rare occurrence that treating the fitness as fertility when it might be viability or both would have very little effect.

The productivity of the incompatible cross compared to P × P gives $V = 0.078$ for 1-day-old males and 0.33 for 4-day-old males. These are comparable to but a little larger than the values of HOFFMANN and TURELLI, and HOFFMANN, TURELLI and SIMMONS, which are near zero for 1-day-old males, 0.12 for 3–4-day-old males and 0.26 for 7–8-day-old males.

When using parameter values in the range of the above in discrete-generation iterations, and 2 or 3 weeks for the generation time, the model indicates generally that the frequencies should accelerate more than the observed values.

However, these populations are age structured and when a very simple age structure model is used a reasonably good fit can be obtained. Little is known of the demography of population cages, so m_x bottle data of GOWAN and JOHNSON (1946) and ROBERTSON and SANGE (1944) were used together with the as-

TABLE 2

Model

Mating ♀ × ♂	Frequency	Offspring × $\bar{W}$ C	P	P_1
C × C	p_1^2	Up_1^2		
P × P	p_2^2		p_2^2	
P_1 × P_1	p_3^2			Up_3^2
C × P	p_1p_2	Up_1p_2		
C × P_1	p_1p_3	Up_1p_3		
P × P_1	p_2p_3		$V(1-L)p_2p_3$	VLp_2p_3
P × C	p_2p_1		$V(1-L)p_2p_1$	VLp_2p_1
P_1 × C	p_3p_1			Up_3p_1
P_1 × P	p_3p_2			Up_3p_2

Where, $p_1 = f(C)$, $p_2 = f(P)$, $p_3 = f(P_1)$

Recurrence equations (1)

$$p'_1 = p_1U\bar{W}^{-1} \quad (1A)$$

$$p'_2 = [p_2 + V(1-L)p_2(1-p_2)]\bar{W}^{-1} \quad (2B)$$

$$p'_3 = [Up_3 + VLp_2(1-p_2)]\bar{W}^{-1} \quad (3C)$$

$$\bar{W} = p_2^2 + (1-p_2)U + p_2(1-p_2)V$$

Approximate equilibrium with small L and the population initiated at $p_3 = 0$

$$p = \frac{1-U}{1-V}$$

Recurrence equations (2) for age structure,

$$p'_1 = (U\tilde{p}_1)\bar{W}^{-1} \quad (2A)$$

$$p'_2 = (\tilde{p}_2\bar{p}_2 + V(1-L)\tilde{p}_2(1-\bar{p}_2))\bar{W}^{-1} \quad (2B)$$

$$p'_3 = 1 - p'_1 - p'_2 \quad (2C)$$

$$\bar{W} = \tilde{p}_2\bar{p}_2 + U(1-\tilde{p}_2) + V\tilde{p}_2(1-\bar{p}_2)$$

where

p'_1, p'_2, p'_3 are in eggs of $t+1$ produced by the three adult age classes at time t.

$\tilde{p}_i \equiv$ mean over three age classes weighted by 0.40, 0.45, 0.15, as explained in the text.

$\bar{p}_i \equiv$ mean over three age classes weighted by 0.55, 0.33, 0.12, as explained in the text.

sumption of 2 weeks' duration for egg to emergence and a maximum of 3 weeks for adult survival. Stable age distribution was assumed with 1-week time intervals. Based on the data of GOWEN and JOHNSON and ROBERTSON and SANGE the following additional assumptions were made. The relative composition of adult age classes are assumed to be 0.55 for adults between emergence and 1 week of age, 0.33 between 1 and 2 weeks of age, and 0.12 between 2 and 3 weeks of age and also, the values used for the females' contribution to eggs, "relative $l_x m_x$," for the same set of ages were 0.40, 0.45 and 0.15, respectively. Assuming the fitness effects are all in the fertility component, the contribution of adults at time, t, to eggs at $t + 1$, was obtained by using the matings in Table 2 such that the contributions of females were weighted by the "relative $l_x m_x$" above after they had mated to males weighted by the age class composition given above. This procedure results in the recurrence equations (2) in Table 2 for eggs at time $t + 1$ from adults at time, t.

There were six age classes: eggs, pupae, newly emerged adults and the three additional adult age classes, each containing the three types C, P and P_I in frequencies p_1, p_2 and p_3 as defined in Table 2. The mating of the adults at time, t, determined the frequencies in the eggs at time $t + 1$, and the other frequencies were moved up one age class for $t + 1$. The frequencies of the C types, p_1, in the three adult age classes were weighted by the age class composition and the resulting mean for each week provided the trajectory to be compared with the data.

Using parameters closer to the Table 1 values rather than to HOFFMANN and TURELLI a reasonable fit can be obtained. Using a fitness of $U = 0.92$, fertility of $V = 0.30$, assuming a negligible standing frequency of young males, and leakage $L = 0.14$ from HOFFMANN and TURELLI the result is the trajectory plotted together with the experimental data in Figure 2A. In Figure 2B is shown the result when assuming an "8-day week" with $U = 0.92$ and $V = 0.30$. The parameters of Figure 2A predict that at the end of the experiment leakage should have resulted in P_I with frequency $p_3 = 0.021$. This agrees well with the finding of two P_I lines out of the 100 isofemale lines tested at the end of the experiment. The unstable point for these parameters is $\hat{p}_1 = 0.114$. The HOFFMANN and TURELLI parameters of $U = 0.781$ and using $V = 0.20$ give an unstable point of $\hat{p}_1 = 0.274$, which is higher than our initial frequencies. It is unfortunate that our initial frequencies were not low enough for our experimental conditions to provide a qualitative test of the model by demonstrating the unstable point. However, the principle reason for publishing these data is to demonstrate that our results can be adequately explained by known fitness effects of the incompatibility system, meaning that there is no need to invoke selection on mtDNA differences. This is in contrast to the work on *Drosophila pseudoobscura* by MACRAE and ANDERSON (1988) where they observed, to use their words, "dramatic mtDNA frequency changes" which they attribute to fitness effects resulting from the interaction of nuclear and mtDNA.

The incompatibility between the two *D. pseudoobscura* subspecies is different from ours in that it results in F_1 male sterility rather than reduced egg hatch. Also, the maternal effect appears not to be transmitted to subsequent generations as in the microorganism in our case (ORR 1989). Other details are discussed by MACRAE and ANDERSON (1990). Nevertheless, it is clear that experiments of this type seeking selection on mtDNA should be done without such complications.

In addition to reporting the results above we take this opportunity for some observations concerning the natural occurrence of the infected individuals, or R types as opposed to W types using the HOFFMANN and TURELLI notation. With such a low unstable point of, in our case, $\hat{p} = 0.11$, or using the HOFFMANN and TURELLI parameters, $\hat{p} = 0.27$, this would not constitute a substantial barrier to invasion and spread of the R types. HOFFMANN and TURELLI suggest that R types may, in fact, be spreading pointing to their finding of low levels of polymorphism with some R types in a W population, but they also found some W types in an R population.

We have some simulation results of migration in a very simple discrete generation model which might suggest how the parameters could be governing this system. There are two populations, one initially composed of R types and the other of W types, which, after mating within the populations, exchange migrants such that each population is composed of a fraction, m, from the other population. These two populations could be regarded as local populations at the border of the R and W ranges.

If m is less than a critical migration rate, m^*, then R will not invade and a stable equilibrium will result in each population. For the HOFFMANN and TURELLI parameters above, $m^* = 0.018$. If m exceeds this value R will invade the W population, but if, for example, $m = 0.01$ there will be a stable equilibrium with 0.04 R types in the W population and 0.01 W types in the R population.

In this system when the fitness, U, decreases and V increases then the critical migration, m^*, increases up to a point (see below). Using the lower 95% confidence limit for the HOFFMANN and TURELLI fitness estimate which is $U = 0.69$ and the higher $V = 0.30$ used earlier, $m^* = 0.055$ and if m is half this value the stable frequency of immigrant types are 0.07 in the W population and 0.07 in the R population. These

appear to be consistent with the HOFFMANN and TURELLI observations.

If we speculate that the fitness might be lower in the natural environment than under laboratory conditions then when U decreases the system shows a qualitative change. If U is lowered to $U = 0.16$, keeping $V = 0.30$, then when $m > m^* = 0.050$ it is the W type which invades the R population. This is so even though in an isolated population these parameters give the unstable point at $\hat{p} = 0.50$ implying ambiguity as to which type will invade. If the fitness is raised to $U = 0.675$ ($V = 0.30$, still) it is approximately at this point that the situation becomes ambiguous as to which type will invade when $m > m^*$. This is close to the lower confidence limit above. Here m^* is a maximum and $\hat{p} = 0.464$ in an isolated population.

This model, as opposed to the one used above to fit the data, we regard as an instructive metaphore for the much more complex processes occurring in natural populations.

Little is known about long distance migration in Drosophila populations, but the fact of the present localized occurrence of R populations, and also the world wide Adh and inversion clines in *D. melanogaster* (OAKSHOTT *et al.* 1982) where no strong selection has been detected suggest that migration is not so great as is commonly believed.

The final answer to these questions concerning the interaction of fitness effects and migration on population dynamics can be investigated directly by monitoring the boundaries between R and W regions which is now being done on the transect between Northern California (W) and Southern California (R) by HOFFMANN and TURELLI.

We wish to thank BRUCE RISKA for helpful discussions about models and NORMAN EATON for writing the computer programs. The experimental work was supported by a grant from the Italian Ministry of Education.

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Communicating editor: J. R. Powell