Dynamics of Cytoplasmic Incompatibility and mtDNA Variation in Natural *Drosophila simulans* Populations

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ABSTRACT

In *Drosophila simulans* a cytoplasmically transmitted microorganism causes reduced egg hatch when infected males mate with uninfected females. The infection is rapidly spreading northward in California. Data on a specific mtDNA restriction site length polymorphism show that changes in the frequency of mtDNA variants are associated with this spread. All infected flies possess the same mtDNA allele, whereas the uninfected flies are polymorphic. Given that both paternal inheritance of the infection and imperfect maternal transmission have been demonstrated, one might expect instead that both infected and uninfected flies would possess both mtDNA variants. Our data suggest that imperfect female transmission of the infection (and/or the loss of the infection among progeny) is more common in nature than paternal transmission. A simple model of intrapopulation dynamics, with empirically supported parameter values, adequately describes the joint frequencies of the mtDNA variants and incompatibility types.

INTRASPECIFIC reproductive incompatibility caused by cytoplasmic factors has been found in five orders of insects (reviewed by STEVENS and WADE 1990). Many cases of incompatibility seem to be caused by *Wolbachia pipientis*, the same intracellular parasite associated with incompatibility between different California strains of *Drosophila simulans* (O'NEILL *et al.* 1992). Strains with the parasite show unidirectional incompatibility when crossed to uninfected strains, and some infected strains show bidirectional incompatibility (O'NEILL and KARR 1990; MONTCHAMP-MOREAU, FERVEUR and JACQUES 1991). However, only two incompatibility types have been identified in California *simulans*: R (originally collected at Riverside) which is infected with Wolbachia, and W (originally collected at Watsonville) which is uninfected (HOFFMANN, TURELLI and SIMMONS 1986; HOFFMANN and TURELLI 1988; LOUIS and NIGRO 1989; BINNINGTON and HOFFMANN 1989; O'NEILL and KARR 1990). In *D. simulans*, the infection is generally transmitted maternally. Types R and W display unidirectional incompatibility in the sense that many of the eggs produced by matings between W (uninfected) females and R (infected) males fail to hatch; whereas, the reciprocal cross and crosses within compatibility types all produce comparable numbers of adult progeny. In polymorphic populations, W females therefore experience a significant source of offspring mortality to which R females are immune. Although this is counterbalanced somewhat by a slight fecundity deficit for R females, the population frequency of the infected type R is generally expected to increase (HOFFMANN, TURELLI and HARSHMAN 1990; *cf.* CASPARI and WATSON 1959; FINE 1978). This has been observed in several natural populations as the infection, initially found in southern California, has spread rapidly northward [TURELLI and HOFFMANN (1991) and data below].

As the infection sweeps through populations, the frequencies of all maternally inherited variants should change. In particular, mtDNA variant(s) initially associated with infected southern California *simulans* might be expected to increase in frequency in unison with type R. This simple prediction is complicated by two facts. First, paternal transmission of the infection has been observed in the laboratory at a frequency of 1–2% (HOFFMANN and TURELLI 1988). Second, although maternal transmission of the infection seems to be perfect in the laboratory, field-collected R females produce at least 1–3% uninfected progeny (HOFFMANN, TURELLI and HARSHMAN 1990). This latter fact has been used to explain the persistence of about 6% uninfected flies in the predominantly infected populations of southern California (HOFFMANN, TURELLI and HARSHMAN 1990). Given these complications, no simple association between incompatibility types and mtDNA variants is expected.

HALE and HOFFMANN (1990) found an association between incompatibility types and mtDNA variants in natural *simulans* populations. Two mtDNA variants, denoted A and B and differing by only a single, easily scored *Hin*fI restriction site change, were particularly

informative. In their 1988 samples, all isofemale lines collected at Davis in northern California were uninfected type W and carried type A mtDNA (or a related type, denoted D). In contrast, all of the infected R lines from the predominantly infected population at Highgrove (adjacent to Riverside) in southern California carried mtDNA type B (see Figure 1). In polymorphic populations at Lake Cachuma and Piru from the Tehachapi transverse range that separates southern from central California, all R flies examined carried B mtDNA, whereas the W flies carried both A and B mtDNA variants. A third incompatibility type, which has been found in island populations from the Pacific and Indian Oceans, shows bidirectional incompatibility with R and has been associated with another mtDNA variant (MONTCHAMP-MOREAU, FERVEUR and JACQUES 1991; also see ROUSSET, VAUTRIN and SOLIGNAC 1992). Our analyses of hundreds of isofemale lines indicate that this third incompatibility type is absent from California.

In the first analysis of mtDNA and incompatibility dynamics, NIGRO and PROUT (1990) monitored frequencies in six laboratory population cages, in which all R flies initially carried one mtDNA type (denoted C) and all W flies carried another (denoted P). After 400 days, the frequency of R had increased in all the cages as expected; and all 32 flies examined that carried C mtDNA were still type R. In contrast, although the P mtDNA flies were initially all uninfected W, after 400 days two out of 68 P mtDNA lines tested were infected type R. These data indicate a low frequency of paternal infection transmission, as demonstrated by NIGRO and PROUT's (1990) mathematical analysis, but suggest perfect maternal transmission. Both results agree with the laboratory analyses of HOFFMANN and TURELLI (1988) and HOFFMANN, TURELLI and HARSHMAN (1990).

Here we report the frequencies of the mtDNA variants described in HALE and HOFFMANN (1990) from several California populations with different R and W frequencies. Of particular interest are the frequencies of mtDNA variants within locally rare incompatibility types and in populations that have undergone recent large changes in incompatibility type frequencies. We use simple models to make inferences concerning the processes underlying these frequency changes in nature.

MATERIALS AND METHODS

Stocks and field collections: Collections in California were generally made in fruit orchards by the methods described in HOFFMANN, TURELLI and HARSHMAN (1990). The collection sites, years, and numbers of isofemale lines whose mtDNA was examined are listed in Table 1. Figure 1 shows the collection sites (numbered as in Table 1) and other locations discussed. All flies were cultured on standard Drosophila medium at 20–23°.

Frequency of incompatibility types: Females from the field were set up individually in vials. We determined the incompatibility types of the resulting isofemale lines according to the methods of HOFFMANN, TURELLI and HARSHMAN (1990).

Frequency of mtDNA types: Mitochondrial DNA was prepared from adults of isofemale lines using a rapid alkaline lysis method modified from TAMURA and AOTSUKA (1988). Live adults (150 mg) were homogenized in 3 ml of chilled buffer (30 mM Tris-HCl, 0.25 M sucrose, 10 mM EDTA, pH 7.5) in a Teflon/glass homogenizer and centrifuged at 1000 × *g* for 1 min at 4°. The supernatant was recentrifuged at 12,000 × *g* (4°) and the mitochondrial pellet suspended in 150 μl of 10 mM Tris, 10 mM EDTA, 0.15 M NaCl, pH 8.0 on ice. We added 300 μl of freshly prepared 0.18 M NaOH containing 1% sodium dodecyl sulfate and mixed by inversion. Tubes were stored on ice for 5 min, before adding 225 μl of ice-cold potassium acetate (3 M potassium, 5 M acetate), vortexing and standing a further 5 min on ice. To the supernatant from a 5-min (4°) 12,000 × *g* spin was added phenol, followed by vortexing and a 5-min spin at room temperature. The aqueous layer was reextracted with phenol-chloroform and precipitated with 2 volumes of ethanol. The pellet after washing with 70% ethanol was vacuum dried and resuspended in 10 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA and stored at −20°.

mtDNA was digested with either *Hin*fI or *Hpa*I (according to the manufacturers protocol), run on 1% agarose gels and stained with ethidium bromide. Lines scored as A or B *Hin*fI pattern types by end-labeling (HALE and HOFFMANN 1990) were correctly identified using this method. Both A and B mtDNA types produce four easily discernable bands. Three bands are common to both types, with approximate sizes 7.4, 3.2 and 1.2 kb. The fourth band is approximately 2.65 kb for type A and 2.8 kb for type B (see Figure 1 of HALE and SINGH 1991).

RESULTS

Table 1 presents the frequency of mtDNA variant A among the W and R lines from various locations, as well as the estimated local frequency of R when the isofemale lines were established. The locations are ordered north to south and the numbers correspond to those in Figure 1. The data from the W and R lines are presented separately to emphasize the differences in their mtDNA composition. There are two notable results. First, although the samples of W lines display a wide range of mtDNA composition, all 92 of the R lines, obtained from the same range of populations, carry type B mtDNA. This corresponds to a 95% upper bound of 0.03 for the frequency of type A mtDNA among R overall (*i.e.*, $(0.97)^{92} = 0.05$). Second, among the W lines, there is a strong correlation between the frequency of A mtDNA and the frequency of the W incompatibility type in the population ($r^2 = 0.986$). This relationship is illustrated in Figure 2, which also presents a predicted relationship derived below. In northern California populations with W at high frequency (Gridley and Davis in 1988), almost all of the W lines contain A mtDNA. In populations that have intermediate frequencies of R due to its recent spread (Escalon, Ivanhoe and Davis in 1991),

TABLE 1

Frequency of mtDNA variant A in populations with different frequencies of infected (R) flies (includes data from HALE and HOFFMANN 1990)

Location	Year	Frequency(R)[a]	W lines			R lines	
			N	Frequency(A)	95% CI[b]	N	Frequency(A)
1. Gridley	1990	0.07	19	0.95	(0.74, 0.999)	3	0.00
2a. Davis	1988	0.00	6	1.00[c]	0.61		
2b. Davis	1991	0.21	24	0.71	(0.49, 0.87)		
3. Escalon	1990	0.29	11	0.72	(0.39, 0.94)	3	0.00
4. Sanger	1989	0.27				23	0.00
5. Ivanhoe	1990	0.81	10	0.20	(0.03, 0.56)	10	0.00
6. Solvang	1990	0.90	3	0.00	0.63	6	0.00
7. Lake Cachuma	1988	0.45	12	0.50	(0.21, 0.79)	9	0.00
8. Piru	1988	0.44	15	0.47	(0.21, 0.73)	13	0.00
9. Highgrove	1989	0.94	15	0.00	0.18	25	0.00

[a] Estimates from 1990 and earlier are based on data in HOFFMANN, TURELLI and HARSHMAN (1990) and TURELLI and HOFFMANN (1991). The 1988 Davis estimate is based on only 12 isofemale lines, all others are based on at least 48.

[b] "Exact" confidence intervals with probability 0.025 in each tail were computed numerically from the binomial distribution. When Frequency(A) equals 0.0 (1.0), the upper (lower) 5% value is given.

[c] This includes one "D" line, closely related to type A [see HALE and HOFFMANN (1990) and HALE and SINGH (1991)].

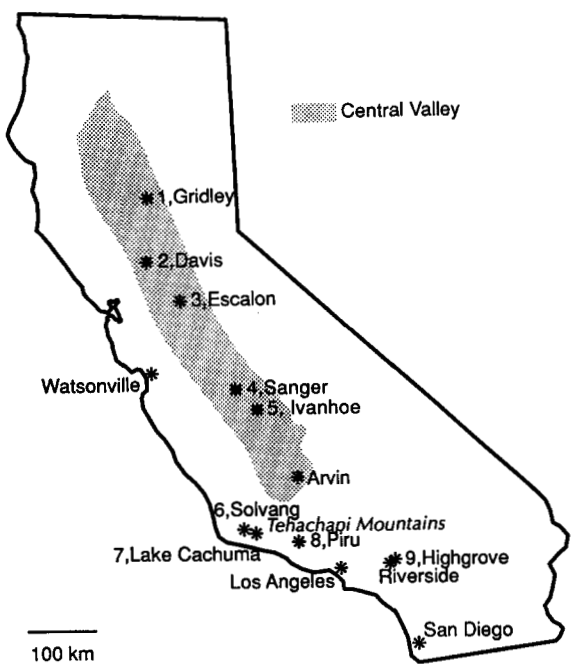

FIGURE 1.—Map of California illustrating the approximate location of our collection sites and other sites discussed in the text. The numbers correspond to Table 1. The Tehachapi range produces the southern boundary of the Central Valley.

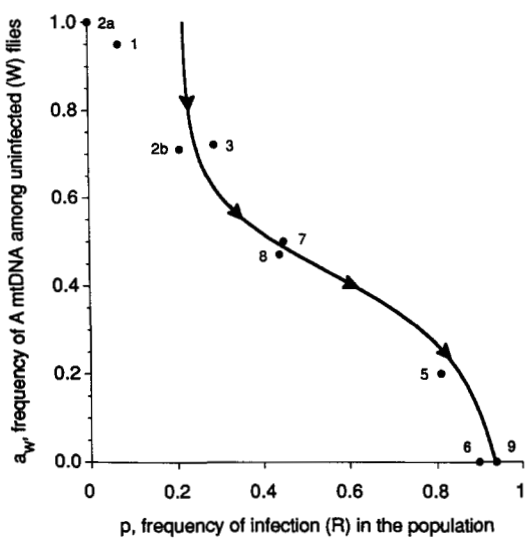

FIGURE 2.—Estimated frequency of type A mtDNA among W flies (a_w) plotted against the frequency of incompatibility type R in the population (p). The data (solid circles) are compared to a theoretical prediction (curve) explained in the text. The arrows on the curve indicate the predicted direction of evolutionary change.

there are intermediate frequencies of the B mtDNA variant among the W lines. The same is true of the Piru and Lake Cachuma populations, which unexpectedly remained highly polymorphic for R and W for some years (HOFFMANN, TURELLI and HARSHMAN 1990). In contrast, in southern California (Highgrove), where incompatibility type R has predominated for at least 6 years, all 15 W flies carried B mtDNA, which was found by HALE and HOFFMANN (1990) to be characteristic of R lines.

ANALYSES

We will first qualitatively analyze our mtDNA data and compare them to previous results, then present a quantitative analysis based on simple models for the joint frequency dynamics of incompatibility types and mtDNA variants.

Qualitative analysis: HALE and HOFFMANN (1990) found that type A mtDNA was prevalent in northern California, whereas type B was prevalent below the Tehachapis in southern California (Figure 1). This fortuitous difference in initial mtDNA composition allows us to evaluate the contribution of migration to increasing the frequency of R in central and northern California and the persistence of W at low frequencies

in southern California. TURELLI and HOFFMANN (1991) interpreted the temporal and spatial increase of R through central and northern California as a Bartonian "wave of advance," in which local selection against R when it is rare is overwhelmed by migration from populations in which R predominates (*cf.* BARTON 1979). An alternative interpretation is that R has increased independently in several populations, with migration playing essentially no role. This might occur, for instance, by frequent horizontal transmission from other infected insect species (O'NEILL *et al.* 1992; ROUSSET, VAUTRIN and SOLIGNAC 1992). If we assume instead that southern California is the source of the infected R flies that are sweeping through central and northern California, we expect that variant B mtDNA will also spread from southern California. This is confirmed by the fact that all of the R flies in central and northern California carry B mtDNA. Thus, migration seems to have played a central role in the spread of R.

Similarly, the persistence of roughly 6% uninfected flies in southern California may be attributable to either recurrent migration from uninfected populations or "curing" of infected flies in nature (HOFFMANN, TURELLI and HARSHMAN 1990). Given that the infection is at a high frequency from Los Angeles south to San Diego, the most likely origin of uninfected flies is central California. However, if the W flies in Highgrove were recent migrants from central California, the data in Table 1 imply that they should carry both A and B mtDNA. Instead all of the W flies in Highgrove carry B mtDNA, as do the R flies. Moreover, the 95% upper confidence limit for the frequency of A mtDNA among W flies in Highgrove (0.18) is much lower than the average A mtDNA frequency among W flies in polymorphic populations to the north. This suggests that most or all of the uninfected flies in Highgrove arose *in situ* via some form of curing.

If initially all infected R flies carried type B mtDNA and all uninfected W flies carried A mtDNA, departures from complete association of R with B would indicate paternal transmission of the infection, imperfect female transmission and/or curing. Paternal transmission would produce R flies with A mtDNA, whereas imperfect female transmission or curing would produce W flies with B mtDNA. In cage populations, NIGRO and PROUT (1990) began with these ideal initial conditions. They found that after 400 days, two out of 34 R lines had the "wrong" mtDNA type and argued that this was consistent with a paternal transmission rate of about 1.4%, as observed by HOFFMANN and TURELLI (1988) in laboratory experiments. The absence of A mtDNA among our 92 R lines suggests a lower paternal transmission rate in nature. NIGRO and PROUT (1990) did not include curing or imperfect maternal transmission in their analysis, because all of the uninfected flies they sampled possessed the mtDNA variant initially associated with their uninfected stock. However, the relationship between the frequency of A mtDNA among our W flies and the frequency of R in the population suggests that as R increases in nature, a larger fraction of the remaining uninfected flies are "cured" descendants of R mothers rather than descendants of the ancestral uninfected population. Some algebra is needed to quantify these processes.

Quantitative analysis: We will begin by generalizing the model presented in HOFFMANN, TURELLI and HARSHMAN (1990) to include mtDNA polymorphism. For algebraic simplicity, this model considers an isolated population, assumes discrete generations, and ignores paternal transmission of the infection (which will be treated subsequently, see Equations 5–7). We will also ignore paternal transmission of mtDNA, which seems to occur at too low a rate in Drosophila (approximately 0.1%) (KONDO *et al.* 1990) to significantly influence our results. Let $H = 1 - s_h$ denote the relative hatch rate from incompatible fertilizations (*i.e.*, fertilizations of W eggs by R sperm), and let $F = 1 - s_f$ denote the relative fecundity of R females. Incompatibility implies $s_h > 0$. The data of HOFFMANN, TURELLI and HARSHMAN (1990) indicate that $s_f > 0$, *i.e.*, Wolbachia reduces R female fecundity, but that the infection does not induce nonrandom mating, affect male mating success, or affect viability in either sex. Thus, we will assume random mating, equal mating success of infected and uninfected males, and no viability selection. Following NIGRO and PROUT (1990), we assume that the mtDNA variants are neutral (*i.e.*, that their fitness effects are far smaller than those associated with the incompatibility system). HOFFMANN, TURELLI and HARSHMAN (1990) observed imperfect transmission of the infection by field-caught females. Therefore, to explain the persistence of uninfected flies in predominantly infected populations, we assume that a fraction μ of the eggs produced by R mothers are W. Alternatively, infected larvae and/or adults may themselves be "cured" to W, for instance by encountering antibiotics in nature (STEVENS 1989; STEVENS and WICKLOW 1992). This slightly alters the dynamics, as discussed below (see Equations 3 and 4). To further simplify the analysis, we will assume that initially all R carry B mtDNA, consistent with the fact that all our R flies had B mtDNA. Given that we are ignoring paternal transmission, this implies that R flies will always carry B mtDNA. Hence, the variables p_t, the frequency of type R among adults in generation t, and $a_{W,t}$, the frequency of mtDNA variant A among W flies, completely describe the joint frequency dynamics of incompatibility and mtDNA types.

Using W_A and W_B to denote uninfected flies with

TABLE 2

Derivation of recursions for infection frequencies and frequencies of the mtDNA variants assuming incomplete maternal transmission and occasional paternal transmission

Mating		Adult progeny × $\bar{W}$ / Mating frequency			
♀ × ♂	frequency	R_A	R_B	W_A	W_B
$R_A \times R$	$(1 - b_R)p^2$	$F[(1 - \mu) + \mu\tau H]$		$F\mu(1 - \tau)H$	
$R_A \times W$	$(1 - b_R)pq$	$F(1 - \mu)$		$F\mu$	
$R_B \times R$	$b_R p^2$		$F[(1 - \mu) + \mu\tau H]$		$F\mu(1 - \tau)H$
$R_B \times W$	$b_R pq$		$F(1 - \mu)$		$F\mu$
$W_A \times R$	pqa_W	$H\tau$		$H(1 - \tau)$	
$W_A \times W$	$q^2 a_W$			1	
$W_B \times R$	$pq(1 - a_W)$		$H\tau$		$H(1 - \tau)$
$W_B \times W$	$q^2(1 - a_W)$				1

Where p = frequency(R), $q = 1 - p$, a_W = frequency of A mtDNA among W flies, qa_W = overall frequency of W with A mtDNA [*i.e.*, frequency(W_A)], b_R = frequency of B mtDNA among R flies, frequency(R_A) = $p(1 - b_R)$, $F = 1 - s_f$ = relative fecundity of R females, $H = 1 - s_h$ = relative hatch rate from incompatible fertilizations (*i.e.*, R sperm and W eggs), μ = fraction of eggs produced by R mothers that are W, τ = fraction of surviving embryos from incompatible fertilizations that are infected (paternal transmission rate), and $\bar{W} = 1 - p[s_f + s_h(q + \mu F p)]$.

the alternative mtDNA genotypes, Table 2 illustrates the consequences of our assumptions, including some generalizations discussed below. Under our current simplifying assumptions, the paternal transmission parameter $\tau = 0$ and $b_R \equiv 1$, so all of the entries involving R_A (infected flies carrying A mtDNA) can be ignored. After some simplification, we obtain

$$p_{t+1} = \frac{p_t(1 - \mu)(1 - s_f)}{1 - s_f p_t - s_h p_t(1 - p_t) - \mu s_h(1 - s_f)p_t^2} \quad (1)$$

and

$$a_{W,t+1} = \frac{a_{W,t}}{1 + \dfrac{\mu(1 - s_f)p_t}{1 - p_t}} < a_{W,t} \quad (2)$$

for $0 < p_t < 1$ and $\mu > 0$.

Equation 1 was used by HOFFMANN, TURELLI and HARSHMAN (1990) to explain the persistence of a low frequency of W in southern California (*cf.* FINE 1978); it reduces to Equation 1A of NIGRO and PROUT (1990) with perfect maternal transmission ($\mu = 0$). Equation 2 shows that as long as both incompatibility types are present, the frequency of mtDNA variant A among W flies will decline. We will now show that for plausible parameter values, this simple model adequately reproduces the empirical relationship between p and a_W in Figure 2.HOFFMANN, TURELLI and HARSHMAN (1990) analyzed Equation 1 and presented parameter estimates based on wild-caught females. Their data suggest that $s_h = 1 - H = 0.2$–0.6, $s_f = 0.0$–0.1, and $\mu \geq 0.01$–0.03. For definiteness, we assume $s_h = 0.45$ and $s_f = 0.05$, which produce dynamics of p_t consistent with those observed in nature (TURELLI and HOFFMANN 1991). For these and similar parameter values, Equation 1 predicts three equilibria: a stable equilibrium at $p = 0$, corresponding to elimination of R; an unstable equilibrium at a low p; and a stable polymorphic equilibrium with R near fixation. If $s_h = 0.45$, $s_f = 0.05$ and $\mu = 0.02$, the unstable equilibrium is $\hat{p} = 0.16$ and the stable polymorphic equilibrium is $\hat{p} = 0.97$. Thus, $\mu = 0.02$ is too low to account for the maintenance of approximately 6% uninfected flies at equilibrium with $s_h = 0.45$ and $s_f = 0.05$. If we equate the long-term frequency of R at Riverside/Highgrove (0.94) to a stable equilibrium for (1) with $s_h = 0.45$ and $s_f = 0.05$, we obtain $\mu = 0.04$, which implies an unstable equilibrium at $p = 0.217$. Although this value for μ exceeds our preliminary estimate, that estimate was conservative because it ignored lines that were difficult to categorize in our initial progeny tests and it did not consider curing of larvae or adults in the field. Next, we determine the implications of these parameter values for mtDNA frequencies.

The frequency of R will increase in an isolated population with $s_h = 0.45$, $s_f = 0.05$ and $\mu = 0.04$, only if $p_0 > 0.217$. We will assume $p_0 = 0.22$. Given our data and those of HALE and HOFFMANN (1990) concerning the prevalence of mtDNA type A in uninfected populations, we also assume that $a_{W,0} = 1$, *i.e.*, initially all W carry A mtDNA. The resulting trajectories for p_t and $a_{W,t}$ are illustrated in Figure 3. Note that $a_{W,t}$ steadily declines as p_t increases, reflecting the fact that more and more of the remaining W flies have R maternal ancestors and hence B mtDNA. The time scale for this change is roughly 50–70 generations, which is likely to be 3–5 years in these populations (TURELLI and HOFFMANN 1991). If we assume that all of our natural populations were sampled at different stages in this evolutionary trajectory, we can use the pairs $(p_t, a_{W,t})$ from Figure 3 to plot a_W *vs.* p. This is the curve in Figure 2, in which the arrows indicate the direction of evolutionary change. Given the number of simplifying assumptions, the agreement

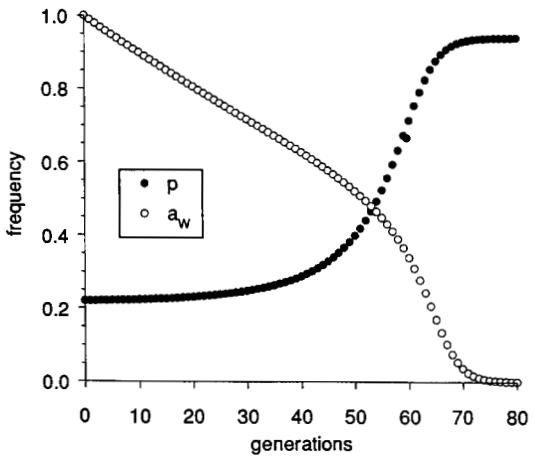

FIGURE 3.—Dynamics of a_W (open circles) and p (closed circles) generated from Equations 1 and 2 with $s_h = 0.45$, $s_f = 0.05$, $\mu = 0.04$, $a_{W,0} = 1$ and $p_0 = 0.22$.

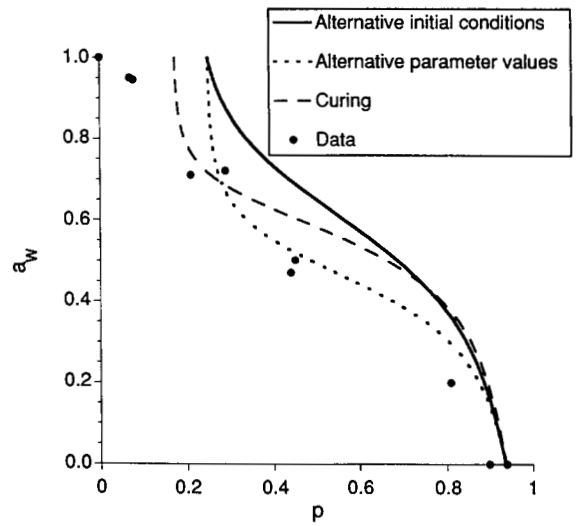

FIGURE 4.—Observed and predicted a_W *vs.* p for different initial conditions and models. The closed circles are the data from Table 1 and Figure 2. The solid curve is generated from Equations 1 and 2 with $s_h = 0.45$, $s_f = 0.05$, $\mu = 0.04$ and $p_0 = 0.25$. The dotted curve is generated by the same equations with $s_h = 0.30$, $s_f = 0.05$, $\mu = 0.02$ and $p_0 = 0.251$. The dashed curve is generated from Equations 3 and 4 with $s_h = 0.45$, $s_f = 0.05$, $\mu_c = 0.024$ and $p_0 = 0.174$. For each curve we assume $a_{W,0} = 1$. See text for additional details.

seems remarkably good for all six values above $p = 0.22$. We can perform a conservative goodness-of-fit test by assuming that p is exactly known in each population and using the theoretical curve in Figure 2 to predict a_W. Only four of the samples are large enough and have expected values of a_W sufficiently far from 0 to justify a G test (SOKAL and ROHLF 1981, Ch. 17). Using the samples 3, 5, 7 and 8 from Table 1, we find $G = 0.69$, which should be approximately distributed as χ^2(d.f. = 4), so that $P > 0.5$.

This simple model cannot predict the relationship between a_W and p for values of p below the unstable equilibrium (0.217), because the model predicts that p should decline from such values. In fact, we know that this prediction is incorrect empirically and is an artifact of ignoring the critical role played by migration in spreading R. A more complete analysis must include population structure and migration. Given the imprecision of our frequency estimates and the apparent inadequacy of the standard diffusion models (which assume a uniformly distributed, persistent population with Gaussian dispersal) to describe the spatial spread of R (TURELLI and HOFFMANN 1991), more sophisticated analyses of these data seem unwarranted. Nevertheless, it is important to understand the robustness of our predictions to different initial conditions and alternative parameter values.

To compensate for ignoring migration and assuming $a_{W,0} = 1$, we have used an initial infection frequency just above the unstable point to produce the theoretical curve in Figure 2. As seen in Figure 3, p increases slowly near the unstable point and there are many generations in which R females are producing B mtDNA-carrying W offspring while the frequency of R changes very little. This yields the rapid predicted decline of a_W with p near the unstable point in Figure 2. In natural populations connected by migration, there will be a phase during which recurrent migration pushes local populations previously monomorphic for W past the unstable equilibrium. During this period, B mtDNA-carrying W individuals will also be introduced. Starting with p near the unstable point and $a_{W,0} = 1$ is intended to mimic this gradual change. (This could also be modeled by starting with a larger p_0 but $a_{W,0} < 1$.) The solid line in Figure 4, obtained with $p_0 = 0.25$ instead of 0.22, shows that the predicted relationship between a_W and p is sensitive to p_0. The new curve fits the data less well (proceeding as above, we obtain $G = 7.88$, d.f. = 4, $P < 0.1$). In particular, it predicts that a_W should be systematically higher than observed. The reason is that for p_0 farther from the unstable point, p changes at roughly the same rate as a_W, producing the lower initial slope seen in Figure 4. A larger p_0 with $a_{W,0} = 1$ corresponds to assuming a massive influx of B mtDNA-bearing R flies into a population initially monomorphic for W with A mtDNA. Such initial conditions might be produced by local extinction and recolonization by long-distance migrants. The resulting qualitative lack of fit displayed in Figure 4 suggests that R may tend to increase locally by a more gradual process.

Estimates of the incompatibility type frequencies in Davis in northern California (Figure 1) from 1990 and 1991 offer additional support for the hypothesis that the frequency of R has increased gradually. The point estimates (95% confidence intervals) and sample sizes are 0.03 (0.004, 0.11) and 62, respectively, for 1990; *vs.* 0.21 (0.12, 0.32) and 71, respectively, for 1991. The observed increase from 3% to 21% is statistically significant ($G = 10.78$, d.f. = 1, $P < 0.01$). Contemporaneous data from Escalon, approximately

100 km south of Davis, show that the large increases in the frequency of R observed at Arvin and Sanger/Ivanhoe (TURELLI and HOFFMANN 1991) are being repeated farther north. The point estimates for p_t (95% confidence intervals) and sample sizes are 0.29 (0.17, 0.44) and 48, respectively, for 1990; *vs.* 0.69 (0.39, 0.91) and 13, respectively, for 1991 ($G = 6.84$, d.f. = 1, $P < 0.01$).

To explore the consequences of alternative parameter values for the theoretical relationship displayed in Figure 2, we need criteria to determine which are plausible. The data of HOFFMANN, TURELLI and HARSHMAN (1990) suggest $s_h = 1 - H = 0.2$–0.6, $s_f = 0.0$–0.1, and $\mu \geq 0.01$–0.03. Additional constraints emerge from infection frequencies in nature. Equation 1 produces a quadratic equation for the unstable and stable polymorphic equilibria (see Equation 3 in HOFFMANN, TURELLI and HARSHMAN 1990). Assuming that 0.94, the long-term frequency of W at Riverside/Highgrove, is a stable equilibrium produces a surface of consistent (s_h, s_f, μ) values. Over the range of empirically supported values, the μ needed to obtain the observed frequency is more sensitive to s_h than s_f (*e.g.*, with $s_h = 0.45$, the required μ increases by only 0.006 with $s_f = 0.1$ *vs.* $s_f = 0$ and similar differences occur for all s_h between 0.2 and 0.6). Thus, we will use only $s_f = 0.05$. Another constraint on the parameter values is imposed by the fact that the infection frequency increased from near 0.3 to just over 0.8 in two widely separated Central Valley populations in about one year (TURELLI and HOFFMANN 1991). By iterating (1), we can determine how many generations this requires for different parameter values. For instance, with $s_h = 0.2$, it takes 21 generations to produce the observed change with $\mu = 0$ and $s_f = 0$; and this increases to 50 generations if $s_f = 0.05$. Given the yearly range of temperatures and fly abundances in the Central Valley, it seems unlikely that more than 20 generations are produced per year and 15 seems more plausible. Combining these constraints derived from models with the data of HOFFMANN, TURELLI and HARSHMAN (1990), we suggest that 0.3 is a reasonable lower bound for a "typical" s_h under field conditions.

Setting $s_h = 0.3$ and $s_f = 0.05$, an imperfect maternal transmission rate of $\mu = 0.02$ suffices to produce a stable equilibrium of (1) at $\hat{p} = 0.94$. The corresponding unstable equilibrium is 0.249. The dotted curve in Figure 4 is the predicted relationship between p and a_W, assuming initial values of $p = 0.251$ and $a_W = 1.0$. The fit to the data is comparable to that obtained with $s_h = 0.45$ and $\mu = 0.04$ ($G = 1.86$, d.f. = 4, $P > 0.5$). These results show that a range of parameter values are consistent with the observed relationship between mtDNA and incompatibility type frequencies. Next, we analyze the effects of changing the model by first considering larval curing instead of imperfect maternal transmission, then incorporating occasional paternal transmission.

Larval curing vs. imperfect maternal transmission: Equation 1 assumes that a fraction μ of the eggs produced by an infected female do not carry the infection and, therefore, are no longer compatible with sperm from R males. This implies that R females, like W females, lose some progeny due to incompatibility. An alternative assumption, suggested by the work of STEVENS (1989) and STEVENS and WICKLOW (1992), is that R larvae and/or adults are occasionally "cured" by encountering antibiotics in nature (or, possibly, continued exposure to high temperatures). Let μ_c denote the curing rate, *i.e.*, the fraction of R larvae and/or adults that become W. Assuming that the adults are sampled after curing and that all other assumptions leading to (1) and (2) hold, we can derive the resulting recursions by slightly altering the expressions in Table 2. As before, we set $\tau = 0$ and $b_R \equiv 1$ to reflect the lack of paternal transmission and the assumption that all R initially carry B mtDNA. Next, replace μ by μ_c and change the last entry in the row corresponding to matings between R_B females and R males from $F\mu_cH$ to $F\mu_c$. This reflects the fact that new W are produced after fertilization, and it increases $\bar{W}$ to $1 - s_f p - s_h p(1 - p)$. With these changes, Equations 1 and 2 are replaced by

$$p_{t+1} = \frac{p_t(1 - \mu_c)(1 - s_f)}{1 - s_f p_t - s_h p_t(1 - p_t)} \tag{3}$$

and

$$a_{W,t+1} = \frac{a_{W,t}}{1 + \dfrac{\mu_c(1 - s_f)p_t}{(1 - s_h p_t)(1 - p_t)}} < a_{W,t} \tag{4}$$

for $0 p_t < 1$ and $\mu_c > 0$.

These equations would also hold if all eggs from R females remain compatible with R sperm even if they do not carry the infection.

Making 0.94 a stable equilibrium of (3) with $s_h = 0.45$ and $s_f = 0.05$ requires $\mu_c = 0.024$. This is only 60% as large as the imperfect transmission rate needed to produce the same equilibrium. Imperfect transmission is less efficient than curing for maintaining uninfected adults, because when the frequency of R is high, most W eggs produced by R mothers will be fertilized by R sperm, so that many of the resulting eggs fail to hatch. In contrast, with larval curing all newly produced W will appear in the adult census.

To produce a prediction analogous to that in Figure 2, note that with $s_h = 0.45$, $s_f = 0.05$ and $\mu_c = 0.024$, the unstable equilibrium is $\hat{p} = 0.172$. Setting $p_0 = 0.174$ produces the predicted relationship between a_W and p shown as a dashed curve in Figure 4. This again adequately fits our data ($G = 3.23$, d.f. = 4, $P > 0.5$).

A model combining both imperfect maternal transmission and larval curing would be intermediate.

Paternal transmission: If paternal transmission of the infection occurs at an appreciable rate, *e.g.*, 1–2%, we would expect to find some R flies carrying A mtDNA, especially in populations in which the frequency of R has recently increased. The absence of such flies suggests that paternal transmission is rare in nature. To quantify this, we must include paternal transmission in our model. Given that all four combinations of incompatibility and mtDNA types will appear, we need a new variable. Let b_R denote the frequency of B mtDNA among R flies. Assuming complete initial association of W with A and R with B, *i.e.*, $a_{W,0} = b_{R,0} = 1$, estimated values of a_W and b_R below one indicate paternal transmission and incomplete maternal transmission, respectively. We will assume incomplete maternal transmission at rate μ and assume that a fraction τ of the surviving offspring produced by fertilizations of W eggs by R sperm carry the infection. Thus, τ denotes the rate of paternal transmission. We now need all of Table 2 to model the resulting dynamics. The most complex expression describes the production of R_A offspring (infected and carrying A mtDNA) from matings between R_A females and R males. This expression is the sum of two terms: the first, $F(1 - \mu)$, involves maternal transmission of the infection (and, hence, compatible fertilization); the second, $F\mu\tau H$, involves W eggs produced by R_A mothers that gain the infection from R sperm (a fraction H survive the incompatible fertilization). The resulting recursions are

$$p_{t+1} = \frac{p_t[(1 - \mu)F + \tau H(1 - p_t + p_t\mu F)]}{1 - s_f p_t - s_h p_t(1 - p_t) - \mu s_h F p_t^2}, \quad (5)$$

$$a_{W,t+1} = \frac{a_{W,t} + \dfrac{(1 - b_{R,t})p_t\mu F}{1 - p_t}}{1 + \dfrac{p_t\mu F}{1 - p_t}}, \quad (6)$$

and

$$b_{R,t+1} = \frac{b_{R,t}(1-\mu)F + \tau H[(1 - p_t)(1 - a_{W,t}) + b_{R,t}p_t\mu F]}{(1 - \mu)F + \tau H(1 - p_t + p_t\mu F)}, \quad (7)$$

with $H = 1 - s_h$ and $F = 1 - s_f$. If $\mu = 0$ and $a_{W,0} = 1$, these are equivalent to Equations 1A–3C of NIGRO and PROUT (1990).

Like Equation 1, Equation 5 produces a quadratic equation for the polymorphic equilibria. For realistic parameter values, the infection will either be lost or reach a stable equilibrium frequency near 1. For $\mu > 0$, $\tau > 0$ and $0 < p_t < 1$, Equations 6 and 7 imply that $a_{W,t+1} < a_{W,t}$ and $b_{R,t+1} < b_{R,t}$ for $a_{W,t} + b_{R,t} > 1$. The frequencies of the neutral mtDNA variants within each incompatibility type will stop changing only when $a_W = 1 - b_R$, *i.e.*, when each mtDNA variant has the same frequency within R and W. The mtDNA frequencies may end up anywhere along the line $a_W + b_R = 1$ depending on the initial conditions. If $a_{W,0} = b_{R,0} = 1$, both frequencies decrease monotonically toward this line.

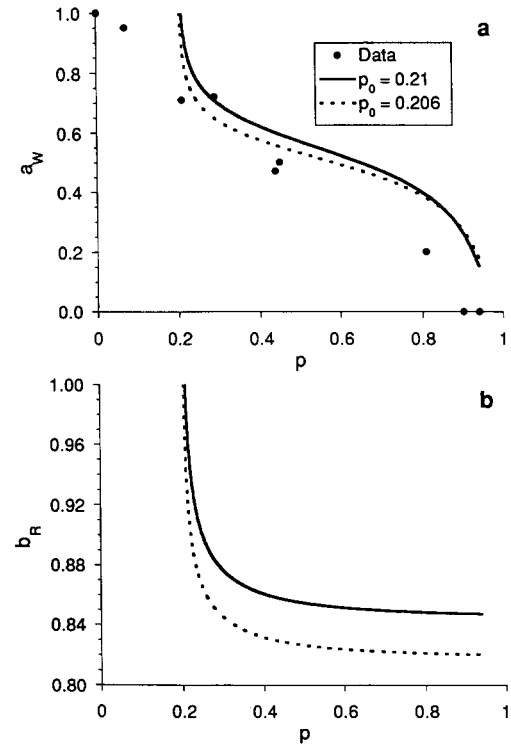

FIGURE 5.—Effects of paternal transmission on (a) a_W *vs.* p and (b) b_R *vs.* p for different initial conditions. The predictions are based on Equations 5–7 with $s_h = 0.45$, $s_f = 0.05$, $\mu = 0.04$, $\tau = 0.01$, $a_{W,0} = b_{R,0} = 1$ and $p_0 = 0.21$ (solid curves) or $p_0 = 0.206$ (dotted curves).

This indicates that quantitative predictions from Equations 5–7 will be sensitive to initial conditions. Nevertheless, by using conservative values, we can make inferences about the rate of paternal transmission in nature. If we use our standard estimates, $s_h = 0.45$, $s_f = 0.05$ and $\mu = 0.04$, and assume a 1% paternal transmission rate ($\tau = 0.01$), Equation 5 produces a stable equilibrium at 0.94 and an unstable equilibrium at 0.203. For the populations in the Central Valley (Sanger, Escalon and Ivanhoe), where we know that p has recently increased, we can reasonably assume $a_{W,0} = b_{R,0} = 1$. Figure 5 shows the resulting predictions for a_W and b_R *vs.* p using two initial conditions, $p_0 = 0.206$ and $p_0 = 0.21$. Clearly p_0 affects the relationship between b_R and p, and the lower initial condition provides a better fit for a_W. We will conservatively assume that if p exceeds 0.25, b_R should be no larger than 0.9. Thus, the probability that all 36 of the R lines examined from Sanger, Escalon and Ivanhoe carry type B mtDNA would be less than $(0.9)^{36} = 0.02$, implying that the paternal transmission frequency in nature is probably less than 1%. If we

assume $a_{W,0} = b_{R,0} = 1$ for the two populations in the Tehachapis (Piru and Lake Cachuma), we can also reject $\tau = 0.005$ (the unstable equilibrium is 0.210; for $p_0 = 0.215$, we expect $b_R < 0.94$ for $p \geq 0.26$; and $(0.94)^{58} = 0.03$). The assumption $a_{W,0} = b_{R,0} = 1$ is consistent with the estimates of a_W in Piru and Lake Cachuma, as shown in Figures 2 and 4. However, given the proximity of these populations to southern California, the W flies there may have been polymorphic for the mtDNA variants before the invasion of R.

Our rejection of $\tau = 0.01$ is robust to using different plausible values of s_h, s_f and μ. Hypotheses concerning τ can be tested further by examining the frequency through time of A mtDNA in W flies from Davis or other locations known to have been initially monomorphic for W and A mtDNA. With paternal transmission, type A mtDNA should persist among W flies as the infection frequency increases to near fixation (compare the theoretical predictions in Figure 5a with Figures 2 and 4).

CONCLUSIONS

Simple models adequately describe the joint dynamics of incompatibility types and mtDNA variants. Our analyses assume that the same parameter values for incompatibility (s_h), fecundity selection (s_f) and incomplete maternal transmission (μ) apply to all of the populations examined. We also assume that in each population where the frequency of R has increased over time, all of the original uninfected flies carried the A mtDNA variant whereas all of the invading infected flies carried the B mtDNA variant (*cf.* HALE and HOFFMANN 1990). Even with this fortuitous initial association, our data have only limited power to distinguish between alternative values for s_h, s_f and μ. However, additional mtDNA and incompatibility type data from populations in central and northern California where R is rapidly spreading will provide increasing resolution.

Although the mtDNA changes that accompany the spread of R are reminiscent of nuclear hitchhiking phenomena (*e.g.*, KAPLAN, HUDSON and LANGLEY 1989), the peculiarities of infection transmission limit this analogy. With both paternal transmission and incomplete maternal transmission of the infection, mtDNA variants would "recombine" with incompatibility types. However, given that paternal transmission seems effectively absent in nature, incomplete maternal transmission acts as a directional force analogous to irreversible mutation. Thus, Equation 2 shows that the mtDNA variant initially associated with R will increase in frequency within uninfected W flies whether or not the frequency of R is increasing.

In contrast to its large effect on mtDNA, the infection's dynamics are unlikely to alter nuclear allele frequencies appreciably, even if the infection is initially completely associated with an allele absent from uninfected populations. Initial disequilibria decay much faster than the slow initial spread of the infection, because the nuclear genome and cytoplasm are unlinked (*cf.* CLARK 1984; ASMUSSEN, ARNOLD and AVISE 1987). This can be verified algebraically by considering the extreme case in which the incompatibility causing infection does not reduce fecundity and is perfectly transmitted maternally. Both factors increase its rate of spread and should maximize the hitchhiking effect. Under these assumptions, the dynamics of the infection frequency p_t follow Equation 1 with $\mu = s_f = 0$. Consider two neutral nuclear alleles, A_1 and A_2. Let $x_{R,t}$ and $x_{W,t}$ denote the frequency of A_1 among R and W adults, respectively, in generation t. Because we assume random mating, the difference in these allele frequencies, $d_t = x_{R,t} - x_{W,t}$, suffices to measure cytonuclear disequilibrium (*cf.* ASMUSSEN, ARNOLD and AVISE 1987). The dynamics of the nuclear alleles are described by

$$x_{R,t+1} = x_{R,t} - (1 - p_t)d_t/2 \text{ and} \tag{8}$$

$$x_{W,t+1} = x_{W,t} + \frac{p_t(1 - s_h)d_t}{2(1 - s_h p_t)}, \tag{9}$$

which imply

$$d_{t+1} = \frac{d_t}{2}\left[1 + p_t\left(1 + \frac{1 - s_h}{1 - s_h p_t}\right)\right]. \tag{10}$$

These equations show that if p_t is initially small and changing slowly, the differences in the allele frequencies between the incompatibility types, d_t, will be roughly halved each generation and $x_{R,t}$ and $x_{W,t}$ will rapidly approach a value near $x_{W,0}$, the initial allele frequency in uninfected flies. Given the differences in the time scales associated with the slow initial increase of p_t and the rapid decrease of d_t, a very accurate approximation for the final allele frequency in the population can be obtained by treating (8) and (9) as linear recursions for $x_{R,t}$ and $x_{W,t}$, with p_t fixed at its initial value. For instance, if $p_0 = 0.05$, $s_h = 0.45$, $x_{R,0} = 0$ and $x_{W,0} = 1$, the population allele frequency begins at 0.95 but both the exact recursions and the linear approximation show that the allele frequencies within each class converge to 0.97 within 10 generations. Thus, we expect that occasional spread of cytoplasmic factors will have little effect on nuclear genomes (*cf.* CASPARI and WATSON 1959).

Among 15 lines of diverse geographic origin outside of California, HALE and HOFFMANN (1990) found a complete association between R and B mtDNA and between W and A mtDNA. Our more extensive data indicate that all infected flies in California carry variant B. Determining the generality of this association will reveal whether this infection has invaded *simulans*

populations only once or multiple times via horizontal transmission from other species (O'NEILL *et al.* 1992). Given the disjunct global distribution of infected populations (HOFFMANN and TURELLI 1988; MONTCHAMP-MOREAU, FERVEUR and JACQUES 1991) and new data comparing *simulans* mtDNA phylogenies to the phylogenies of their associated Wolbachia strains (ROUSSET, VAUTRIN and SOLIGNAC 1992), multiple invasions seem possible. However, the fact that all R flies in our samples carry the same mtDNA allele indicates that horizontal transmission of the infection within *simulans* is quite rare in nature.

The data of HOFFMANN, TURELLI and HARSHMAN (1990) suggested that the incompatibility system behaves differently in the field than the laboratory. In particular, the level of incompatibility (s_h) is lower in the field, and maternal transmission of the infection seems less reliable. HOFFMANN, TURELLI and HARSHMAN (1990) hypothesized that this may be caused by lower intracellular Wolbachia densities under field conditions, and this can soon be tested using molecular quantification techniques (T. L. KARR and S. L. O'NEILL, personal communication). The analysis presented here suggests another discrepancy between laboratory and field data. HOFFMANN and TURELLI (1988) and NIGRO and PROUT (1990) both found evidence for rare paternal transmission in the laboratory. Our mtDNA data suggest that it is less common in nature, as might be expected if Wolbachia densities are lower under field conditions.

The possibility that mtDNA variants can spread as a consequence of cytoplasmic incompatibility needs to be considered when patterns of mtDNA variation are interpreted (HALE and HOFFMANN 1990; MONTCHAMP-MOREAU, FERVEUR and JACQUES 1991). When different populations have similar mtDNA variants, it is normally assumed that they have originated from the same ancestral population which has undergone a founder event or has persisted at a small population size. In *D. simulans*, surveys of mtDNA variation have indicated that populations can be divided into three major mitochondrial types with little variation within each type (BABA-AISSA *et al.* 1988; HALE and SINGH 1991). In the absence of much nuclear differentiation between *D. simulans* populations, this finding has been interpreted as a consequence of the recent spread of *D. simulans* with each major type representing a founder event. However, it is clear from our study that mtDNA variation within and between populations can be drastically reduced as cytoplasmic incompatibility factors spread. Patterns of mtDNA variation in this species and other insects should therefore be interpreted cautiously in the absence of information on incompatibility.

Our data indicate that an mtDNA variant has spread rapidly as a consequence of cytoplasmic incompatibility. In fact, any cytoplasmic variant associated with the infection is expected to spread rapidly. Cytoplasmic incompatibility therefore provides a mechanism for introducing cytoplasmic factors into natural populations. This may eventually be useful for introducing deleterious factors into pest insect populations (*cf.* CURTIS 1976, 1992). Of particular interest are cytoplasmic factors that decrease fitness under specific conditions that can be imposed once factors occur at an appreciable frequency. Possibilities include mitochondria or viruses causing susceptibility to chemicals, or cytoplasmic factors (including genetically engineered Wolbachia) decreasing resistance to temperature extremes.

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