

Geographic Distribution and Inheritance of Three Cytoplasmic Incompatibility Types in *Drosophila simulans*

Catherine Montchamp-Moreau,^{*1} Jean-François Ferveur[†] and Micheline Jacques^{*}

^{*}CNRS UA 693 Dynamique du Génome et Evolution, Université Paris VI, 75252 Paris Cedex 05, France, and

[†]Laboratoire de Biologie et Génétique Evolutives, CNRS, 91198, Gif sur Yvette, France

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ABSTRACT

Wolbachia-like microorganisms have been implicated in unidirectional cytoplasmic incompatibility between strains of *Drosophila simulans*. Reduced egg eclosion occurs when females from uninfected strains (type W) are crossed with males from infected strains (type R). Here we characterize a third incompatibility type (type S) which is also correlated with the presence of Wolbachia-like microorganisms. Despite the fact that the symbionts cannot be morphologically distinguished, we observed complete bidirectional incompatibility between R and S strains. This indicates that the determinants of incompatibility are different in the two infected types. S/W incompatibility is unidirectional and similar to R/W incompatibility. A worldwide survey of *D. simulans* strains showed that type S incompatibility was found only in insular populations which harbor the mitochondrial type SiI. Both W and R types were found among mainland and island populations harboring the worldwide mitochondrial type SiII. Type S incompatibility could be involved in the reinforcement of the geographical isolation of SiI populations.

CYTOPLASMIC incompatibilities have been described in several orders of insects, and in many cases have been found to be related to endosymbiosis (reviewed in HOFFMANN, TURELLI and SIMMONS 1986; BREUWER and WERREN 1990). They have been extensively studied in the mosquito *Culex pipiens*, which exhibits multiple systems of unidirectional and bidirectional incompatibilities, maternally inherited, between or within populations (LAVEN 1957; reviewed in SUBBARAO 1982). These incompatibilities are correlated with the systematic presence of a rickettsial symbiont, *Wolbachia pipientis*, in the germinal cells of the mosquitoes (YEN and BARR 1973). There are no naturally uninfected populations of *C. pipiens*.

In *D. simulans* unidirectional cytoplasmic incompatibility was first reported between Californian strains (HOFFMANN, TURELLI and SIMMONS 1986). Incompatibility occurs when males from infected populations (type R) are crossed with females from uninfected populations (type W). Mating and oviposition occur normally, but a large proportion of eggs fail to hatch. The reciprocal cross is compatible as are crosses between type-R strains and between type-W strains. These two types of populations are widely spread across the world (HOFFMANN and TURELLI 1988). W/R incompatibility is correlated in R strains with the presence of cytoplasmic microorganisms which are morphologically indistinguishable from *W. pipientis* (LOUIS and NIGRO 1989; BINNINGTON and HOFFMANN

1989). More recently, an Hawaiian strain of *D. simulans* containing microorganisms was found to be bidirectionally incompatible with a type R strain (O'NEILL and KARR 1990). This strain probably belongs to the new incompatibility type we report here and propose to call type S, since our reference strain comes from the Seychelles archipelago.

MATERIALS AND METHODS

***D. simulans* strains:** The strains used are listed in Table 1. Most of them originate from several wild-caught females; four of them are isofemale lines. Our reference strains were Seychelles-81 (S) and Nasr'allah (W). Riverside (R) and Watsonville (type W), which are used as reference strains in the R/W incompatibility system, were kindly provided by M. TURELLI.

Incompatibility tests: Tests were performed at 25°. For each mass cross, 30 4-day-old virgin females were allowed to mate for 24 hr with 40 2-day-old virgin males, in a bottle containing standard medium. Individual tests were performed with a single pair of flies in a vial, in the same experimental conditions. In all cases, flies were transferred to oviposit on fresh medium consisting of agar (5%) and saccharose (0.5%) supplemented with vinegar, fresh yeast and animal charcoal powder. After 12–24 hr the adults were discarded. Unhatched eggs were counted at least 24 hr after removal of the adults. The percentage of unhatched eggs was used as a quantitative measure of incompatibility between strains or individuals. Crosses with more than 80% unhatched eggs ($Uh > 80\%$) were considered as incompatible, and those with less than 20% unhatched eggs ($Uh < 20\%$) as compatible. Egg counting in successive generations was carried out as each generation was produced. Control crosses with reference strains were simultaneously observed at each generation.

¹ To whom all correspondence should be addressed.

TABLE 1
Description of strains

| Origin and name (location) | Founder female(s) | Incompatibility type | Collection year | References ^a |
|-----------------------------|-------------------|----------------------|-----------------|-------------------------|
| Europe | | | | |
| La Sirole (France) | 50 | W | 1983 | 3 |
| Bretagne (France) | 15 | W | 1989 | 3 |
| Cagnano (France) | 30 | R | 1989 | 3 |
| Tuscany = C (Italy) | 7 | R | ? | 1 |
| P (Italy) | ? | W | ? | 1 |
| Africa | | | | |
| Nasr'allah (Tunisia) | 50 | W | 1983 | 3 |
| Yaoundé (Cameroon) | 50 | W | 1974 | 3 |
| Loua (Congo) | >30 | R | 1989 | 3 |
| South Africa | >30 | R | 1987 | 3 |
| Kenya? | 1 | W | 1982 | 2 |
| America | | | | |
| Porto Alegre (Brazil) | >30 | R | 1982 | 3 |
| Davis (California) | 12 | W | 1988 | 2 |
| Watsonville (California) | 30 | W | 1984 | 2, 3 |
| Riverside (California) | 30 | W | 1984 | 2, 3 |
| Highgrove (California) | 18 | R | 1985 | 2 |
| Death Valley (California) | 20 | W | 1986 | 2 |
| Beltsville (Maryland) | 1 | R | 1985 | 2 |
| Morven (Georgia) | 1 | W | 1984 | 2 |
| Belmont (Maine) | 8 | W | 1982 | 2 |
| Frisco (North Carolina) | 6 | W | 1985 | 2 |
| Ottawa (Canada) | 1 | R | 1985 | 2 |
| Australia | | | | |
| Sydney | 30 | W | 1982 | 2, 3 |
| Melbourne | 25 | W | 1985 | 2 |
| Townsville | 25 | W | 1985 | 2 |
| Atlantic Ocean | | | | |
| Teneriffe (Canary Islands) | >30 | W | 1986 | 3 |
| Pacific Ocean | | | | |
| Japan | ? | W | ? | 3 |
| Hawaii 1 (USA) | 1 | S | 1985 | 3 |
| Hawaii 4 (USA) | 1 | S | 1985 | 3 |
| Moorea 1 (French Polynesia) | 1 | S | 1986 | 3 |
| Moorea 8 (French Polynesia) | 1 | S | 1986 | 3 |
| Nouméa (New Caledonia) | 28 | S | 1989 | 3 |
| Amieu (New Caledonia) | 8 | S | 1989 | 3 |
| Karaka (New Caledonia) | 5 | S | 1989 | 3 |
| Roussette (New Caledonia) | 12 | S | 1989 | 3 |
| Monirange (New Caledonia) | 14 | S | 1989 | 3 |
| Indian Ocean | | | | |
| Marsantsetra (Madagascar) | 12 | W | 1987 | 3 |
| Ranomafana (Madagascar) | 20 | W | 1987 | 3 |
| Saint Denis (Reunion) | 35 | S | 1989 | 3 |
| Saint Gilles (Reunion) | 26 | S | 1990 | 3 |
| Seychelles 81 (Mahe) | 30 | S | 1981 | 3 |
| Seychelles 85 (Mahe) | 40 | S | 1985 | 3 |

^a 1, NIGRO and LOUIS (1989); 2, HOFFMAN and TURELLI (1988); 3, strains tested in this paper.

Mitochondrial DNA (mtDNA) extracts: For each strain, about 3000 virgin eggs were pooled and mtDNA was extracted following the protocol given in AUBERT (1990).

RESULTS

S incompatibility type in *D. simulans*: Incompatibility was first observed in a cross between Nasr'allah females and Seychelles-81 males (Table 2): mating and oviposition occurred normally but a large percentage of eggs did not hatch and became brown

within a few days, showing that they had been fertilized but had subsequently died. Unhatched eggs were dechorionated and then observed: some did not show any cellularization, while others showed later signs of development (*i.e.*, germ band elongation, tracheae, muscular movements) with varying levels of disorganization. The sex ratio among surviving F₁ adults was not affected (data not shown). In the reciprocal cross (Seychelles-81 females × Nasr'allah males) the percentage of unhatched eggs was very low and not

TABLE 2
Incompatibility between reference strains

| Females | Males | | |
|---------------|------------|---------------|-----------|
| | Nasr'allah | Seychelles-81 | Riverside |
| Nasr'allah | 4.0 | 88.9 | 99.5 |
| Seychelles-81 | 2.9 | 17.5 | 99.1 |
| Riverside | 12.5 | 95.6 | 18.2 |
| Watsonville | 4.2 | 97.0 | 95.6 |

Data given are percentages of unhatched eggs (*Uh*) per cross. Number of eggs for each cross 189 < *N* < 215.

TABLE 3
Effect of tetracycline on incompatibility

| Females | Males | | | |
|---------|-------|------|------|-----|
| | S | | S-TC | |
| | 1 | 2 | 1 | 2 |
| W | 78.1 | 81.1 | 0.6 | 3.0 |
| S | 14.1 | 15.0 | 6.3 | 2.5 |
| S-TC | 84.9 | 79.7 | 5.5 | 4.3 |

W = Nasr'allah, S = Seychelles-81, S-TC = Seychelles-81 reared for three generations on medium with 0.03% tetracycline-HCl Sigma (HOFFMANN, TURELLI and SIMMONS 1986) and then for at least two generations on standard medium. Data given are percentages of unhatched eggs (*Uh*) per cross. Number of eggs for each cross 162 < *N* < 604.

statistically different from that obtained within the Nasr'allah strain: the incompatibility thus appeared to be unidirectional. The percentage of unhatched eggs was significantly higher within the Seychelles-81 strain than within Nasr'allah ($\chi^2 = 12.29$, d.f. = 1, *P* < 0.001).

Seychelles-81 and Nasr'allah flies were crossed with Riverside (type R, infected) and Watsonville (type W, uninfected), reference strains in the R/W incompatibility system (HOFFMANN, TURELLI and SIMMONS 1986). Nasr'allah behaved as a type W strain and was used as our W reference strain in subsequent experiments. Crosses between Riverside and Seychelles-81 were bidirectionally incompatible (Table 2). Seychelles-81 males were equally incompatible with R (Riverside) and W (Nasr'allah and Watsonville) females while Seychelles-81 females were as incompatible as W females (Nasr'allah and Watsonville) with R males. The infected strain Seychelles-81 clearly belongs to a third incompatibility type in *D. simulans*, which we call type S.

Properties and inheritance of S incompatibility: Tetracycline treatment showed no significant effect on the incompatibility characteristics of the W strain (data not shown) but important changes were found in treated S flies (S-TC) which became compatible with W flies and unidirectionally incompatible with untreated S flies (Table 3). This suggests that a microorganism in the S strain is responsible for the S type incompatibility.

TABLE 4
Inheritance of incompatibility between Seychelles-81 and Nasr'allah and male effect

| Cross # (Female × Male) | <i>Uh</i> (%) | | Male age effect |
|------------------------------|-------------------|-------------------|-----------------|
| | Two-day-old males | Six-day-old males | |
| Parental crosses | | | |
| 1 W × W | 4.7 | 5.5 | |
| 2 S × S | 15.5 | 10.4 | |
| 3 W × S | 82.8 | 54.5 | *** |
| 4 S × W | 2.3 | 1.9 | |
| F ₁ females tests | | | |
| 5 WS × S | 91.8 | 62.1 | *** |
| 6 SW × S | 18.7 | 2.8 | |
| 7 WS × W | 0.6 | 1.3 | |
| 8 SW × W | 1.0 | 1.7 | |
| F ₁ males tests | | | |
| 9 S × WS | 3.4 | 1.5 | |
| 10 S × SW | 0.6 | 4.1 | |
| 11 W × WS | 4.2 | 11.3 | |
| 12 W × SW | 38.6 | 33.2 | NS |
| F ₂ females tests | | | |
| 13 (WS) S × S | 99.5 | 66.7 ^a | *** |
| 14 (WS) W × S | 97.0 | 60.7 | *** |
| 15 (SW) S × S | 4.0 | 3.0 | |
| 16 (SW) W × S | 14.5 | 1.0 | |
| F ₂ males tests | | | |
| 17 W × (WS) S | 1.0 | 5.5 | |
| 18 W × (WS) W | 9.5 | 8.5 | |
| 19 W × (SW) S | 51.0 | 27.4 ^b | *** |
| 20 W × (SW) W | 63.0 | 36.5 | *** |
| Control cross | | | |
| 21 W × S | 77.5 | 46.0 | *** |

W = Nasr'allah, S = Seychelles-81; the first letter indicates cytotypic. Data given are percentages of unhatched eggs (*Uh*) per cross. Number of eggs for each cross 169 < *N* < 359, except (a) = 114 and (b) = 117. χ^2 with Yates' correction test for difference in *Uh* (%) between 2-day-old and 6-day-old males: *** = *P* < 0.001, NS = nonsignificant. The control cross (#21) was performed at the end of the experiment.

Four successive generations of crosses between W (Nasr'allah) and S (Seychelles-81) flies were set up and eggs from these crosses were scored (Table 4). In all the incompatible crosses but one (#12), a significant decrease in the level of incompatibility was observed between 2-day-old males and 6-day-old males. A similar male age effect has been observed with R males (HOFFMANN, TURELLI and SIMMONS 1986).

Inheritance of incompatibility traits was estimated from crosses involving 2-day-old males. Incompatibility occurred between S males mated with hybrid females bearing a W cytotypic (#5, 13 and 14), and between W females mated with hybrid males bearing an S cytotypic (#12, 19 and 20). Thus the W and S incompatibility types were maternally inherited. Hybrid S × W males with an S cytotypic (#12, 19 and 20) were significantly less incompatible with W females than parental S males (#3) ($18.8 < \chi^2 < 79.2$, d.f. = 1, *P* < 0.001). This change suggests that a possible paternal effect (due to W paternal factors or addi-

TABLE 5
Effect of parental factors on the incompatibility levels

| Tested strains | Crossed with females | | Crossed with males | | Intrastrain crosses | |
|---------------------------------|----------------------|-------|--------------------|-------|---------------------|------|
| | W | S | W | S | | |
| Backcross WS { | 1 | 0.0 | 10.1 | 1.5 | 77.5 | 1.5 |
| | 2 | 3.0 | 6.5 | 4.0 | 84.0 | 1.9 |
| Backcross SW { | 1 | 32.3 | 4.5 | 5.4 | 72.5 | 31.9 |
| | 2 | 34.0 | 5.7 | 2.0 | 70.5 | 27.8 |
| Control crosses (female × male) | W × W | S × S | S × W | W × S | | |
| | 0.9 | 32.0 | 2.0 | 78.5 | | |

Two replicates (1 and 2) of 30 W females were mated with 40 S males, and at least 30 hybrid females were successively backcrossed to 40 W males for up to nine generations; the resulting strains were called backcross WS. S females were mated with W males, and female offspring were backcrossed to W males for up to nine generations; the resulting strains were called backcross SW. W = Nasr'allah, S = Seychelles-81. Data given are percentages of unhatched eggs (*Uh*) per cross. Number of eggs for each cross $182 < N < 220$. Control crosses were simultaneously tested with the backcross strains.

tional interactions) might modify the incompatibility level of such males, since the incompatibility observed between S and W parental strains remained constant during the course of the experiment.

The role of paternal factors in incompatibility was further shown by reciprocal backcross experiments (Table 5). W type incompatibility is stably inherited through the W cytotypic (WS backcross flies behaved like W flies). Conversely the reciprocal backcross SW strains revealed a paternal (W) inheritance of incompatibility type: SW females were highly incompatible with S males ($Uh = 72\%$), while SW males were only moderately incompatible with W females ($Uh = 33\%$). High levels of incompatibility of SW and W females, both crossed with S males, were not significantly different (χ^2 with Yates' correction = 3.25, $P = NS$). The level of SW males remained significantly higher than that of W males, both crossed with W females ($\chi^2 = 82$, $P < 0.001$). This suggests that the S incompatibility type, which is partially inherited via the S cytoplasm, can be substantially altered by interactions with W paternal factors.

In order to understand the variation of incompatibility level within the S strain and between S and W strains, we scored hatchability in individual cross progenies (Figure 1). Percentages of unhatched eggs, generally low, varied among the progenies of S × S crosses (Figure 1a) implying a substantial intrastrain variation for factor(s) causing S-type incompatibility in either or both sexes. Comparison of this figure with that of the compatible S × W crosses (Figure 1b) showed that about 25% of the crosses within the S strain were moderately incompatible. Crosses between W females and S males also showed a wide distribution varying from intermediate to high incompatibility levels (Figure 1c). One W × S cross was compatible. The compatible female was recrossed with another S male and gave 45% unhatched eggs. No further compatible

W × S cross was observed in subsequent experiments.

Geographical survey of *D. simulans* strains: The 27 strains noted in Table 1 were reciprocally crossed with the W (Nasr'allah), R (Riverside) and S (Seychelles-81) reference strains. Intrastrain egg hatchability was also measured (Table 6). A first group of strains showed unidirectional incompatibility with R and S flies and compatibility with W flies. These strains, which showed a worldwide distribution, were classified as W-type strains (Table 6A). Four other strains, found in various locations, were compatible with R flies, incompatible with W females and bidirectionally incompatible with S flies. They were classified as R-type strains (Table 6B). Ten remaining strains were considered as S-type strains because they were bidirectionally incompatible with R flies, and males were incompatible and females compatible with W flies (Table 6C). S-type strains were only found in the Seychelles, New Caledonia, French Polynesia and Hawaiian islands.

Percentages of unhatched eggs resulting from intrastrain crosses were significantly lower in W-type strains (4.8 ± 0.8) than in S-type strains (17.4 ± 2.2) and in R-type strains (14.9 ± 2.9) (Mann and Whitney rank-sum test, respectively $U = 10$ and 3 , $P < 0.01$). In addition, S-type females were more compatible with W males than with their own type males (Wilcoxon signed rank test, $T = 0$, $n = 11$, $P < 0.01$). Both observations suggest partial incompatibility within S- and R-type strains.

Flies of the 11 S-type strains showed differences in their degree of incompatibility when crossed with S males and with W females (Table 6C). The correlation between these two traits was negative and significant (Spearman rank correlation, $r_s = -0.745$, $n = 11$, $P < 0.025$). S type strains females showed variable levels of incompatibility when crossed with S males ($Uh = 6.5-70.2\%$). Males of all S-type strains, except Karaka,

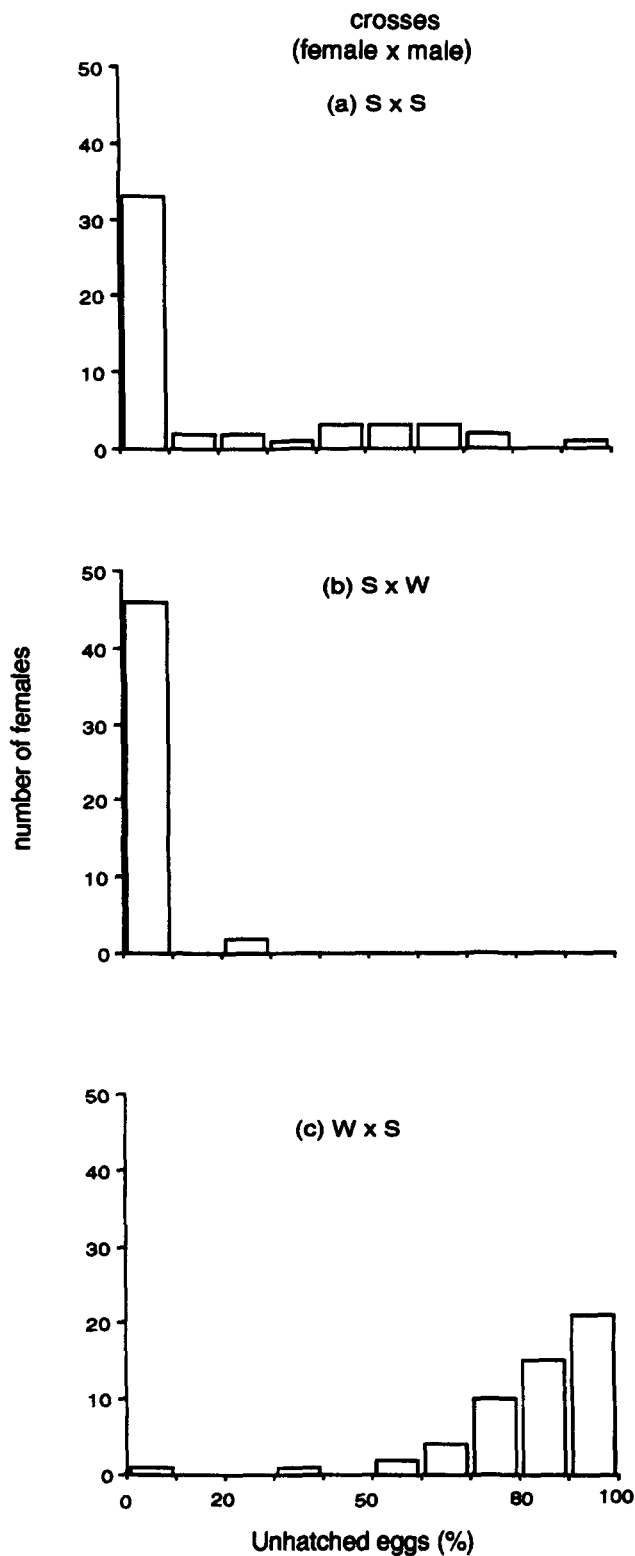


FIGURE 1.—Distribution of the percentage of unatched eggs laid by each female in the following crosses: (a) Seychelles-81 females \times Seychelles-81 males (S \times S), (b) Seychelles-81 females \times Nasr'allah males (S \times W), (c) Nasr'allah females \times Seychelles-81 males (W \times S). Mass mating (60 females and 80 males) was performed according to the standard protocol. Each female was then introduced for 2 days into a separate vial for individual measures of the percentage of unatched eggs. Females which laid less than 30 eggs were discarded.

were incompatible with W females ($Uh > 68\%$). This suggests a high frequency of infected males among these strains at the time of our experiments but gives no information about the frequency of infected flies in natural populations.

Karaka flies exhibited an intermediate phenotype: males were only moderately incompatible with W females ($Uh = 41\%$), and females were markedly incompatible with S males ($Uh = 70.2\%$). Flies of this strain were individually crossed with W females and with S males; intrasrain crosses were also made (Figure 2). Only 56% of Karaka males were highly incompatible, while the rest showed low or moderate incompatibility with W females (Figure 2a). 75% of crosses between Karaka females and S males were incompatible (Figure 2b). Of Karaka intrasrain crosses, 32% were highly incompatible and 41% were completely compatible; the remaining crosses showed intermediate incompatibility (Figure 2c). The Karaka strain was created from five isofemale lines which were pooled a few weeks before testing. Variability in incompatibility shown by Karaka might be due to a naturally occurring polymorphism for factors controlling incompatibility.

This hypothesis could also explain the peculiar behavior of isofemale lines from Hawaii and Moorea. Males from these lines clearly inherited the S type-like infection from their maternal ancestor while females were up to 70% incompatible with S males despite the fact that they probably carry S type-like microorganisms. Other strains from French Polynesia (Tahiti) behaved similarly (data not shown).

The geographical distribution of the three incompatibility types was compared with that of the three mtDNA types in this species (SiI, SiII and SiIII) which have been defined according to their restriction pattern (BABA-AÏSSA and SOLIGNAC 1984) (Figure 3). The distributions of the S incompatibility type and SiI mtDNA type are virtually identical while both R and W types occur within the range of the SiII mitochondrial type. The 11 S-type strains described in Table 6C were analyzed for their mitochondrial DNA profile. DNA from virgin eggs was digested with the endonuclease *HpaI* which produces pattern differences between the three mtDNA types (SOLIGNAC and MONNEROT 1986). After electrophoresis and ethidium bromide coloration all strains showed the pattern of bands which characterize SiI mtDNA. Thus S incompatibility type appears strictly associated with the SiI mitochondrial type.

DISCUSSION

R and S type *D. simulans* strains are bidirectionally incompatible. S/W incompatibility exhibits the same main features as the R/W incompatibility described by HOFFMANN, TURELLI and SIMMONS (1986): both

TABLE 6
Test of strains from various geographical origins

| Tested strains | Intrastrain cross | Crossed with females | | | Crossed with males | | |
|-------------------|-------------------|----------------------|------------|-------------------|--------------------|-------------------|------------|
| | | W | R | S | W | R | S |
| A. W-type strains | | | | | | | |
| Nasr'allah | 4.0 | 4.0 | 12.5 | 2.9 | 4.0 | 99.5 | 88.9 |
| La Sirole | 2.3 | 7.1 | 7.9 | 5.8 | 2.9 | 99.4 | 73.1 |
| Bretagne | 2.8 | 1.0 | 1.4 | 2.5 | 3.5 | 90.3 | 81.2 |
| Tenerife | 10.3 | 5.7 | 6.7 | 4.1 | 8.9 | 98.8 | 71.3 |
| Yaoundé | 3.4 | 8.0 | 11.1 | 6.6 | 2.5 | 96.6 | 91.3 |
| Saint Denis | 6.1 | 2.5 | 3.0 | 4.1 | 1.6 | 95.7 ^a | 73.2 |
| Saint Gilles | 3.9 | 8.9 | 7.9 | 5.0 | 5.0 | 93.8 | 75.0 |
| Marsantsetra | 0.4 | 10.1 | 11.8 | 7.1 | 2.6 | 84.6 ^b | 74.4 |
| Ranomafana | 6.2 | 3.2 | 7.3 | 4.3 | 1.0 | 84.2 | 78.6 |
| Sydney | 8.0 | 9.5 | 11.5 | 1.6 | 1.4 | 89.0 | 80.0 |
| Japan | 5.7 | 9.1 | 8.6 | 4.0 | 9.2 | 96.8 | 88.2 |
| Mean ± SE | 4.8 ± 0.8 | 6.3 ± 1.0 | 8.2 ± 1.1 | 4.4 ± 0.5 | 3.9 ± 0.8 | 93.5 ± 1.7 | 79.6 ± 2.1 |
| B. R-type strains | | | | | | | |
| Riverside | 18.2 | 99.5 | 18.9 | 99.0 | 12.5 | 18.2 | 95.6 |
| Porto Alegre | 23.0 | 99.1 | 13.6 | 100.0 | 9.4 | 9.6 | 70.5 |
| Cagnano | 16.7 | 99.0 | 15.6 | 95.7 | 3.4 | 6.9 | 74.2 |
| Loua | 8.9 | 98.8 | 8.7 | 96.2 | 2.0 | 24.4 | 90.3 |
| South Africa | 7.9 | 84.4 | 24.9 | 78.4 | 4.9 | 4.6 | 80.3 |
| Mean ± SE | 14.9 ± 2.9 | 96.2 ± 2.2 | 16.3 ± 2.7 | 93.9 ± 3.9 | 6.4 ± 2.0 | 12.7 ± 2.2 | 82.2 ± 4.7 |
| C. S-type strains | | | | | | | |
| Seychelles-81 | 17.5 | 78.5 | 95.6 | 17.5 | 2.9 | 98.1 | 17.5 |
| Nouméa | 20.0 | 91.5 | 93.8 | 16.4 | 8.2 | 81.4 | 14.7 |
| Amieu | 1.8 | 95.3 | 91.3 | 23.9 | 0.5 | 85.3 | 6.5 |
| Karaka | 27.9 | 41.0 | 60.5 | 14.5 ^c | 3.3 | 84.7 | 70.2 |
| Roussette | 15.4 | 90.5 | 83.1 | 15.0 | 2.0 | 89.0 | 15.4 |
| Monirange | 21.1 | 84.1 | 85.3 | 11.1 | 3.9 | 80.2 | 30.8 |
| Moorea 1 | 20.1 | 81.7 | 83.1 | 9.1 | 7.8 | 100.0 | 66.0 |
| Moorea 8 | 26.0 | 69.7 | 71.8 | 8.0 | 6.4 | 99.0 | 52.4 |
| Hawaii 1 | 13.7 | 87.6 | 90.2 | 15.3 | 3.1 | 99.5 | 54.9 |
| Hawaii 4 | 11.5 | 68.5 | 71.7 | 8.2 | 9.0 | 100.0 | 68.4 |
| Seychelles-85 | 16.5 | 80.7 | 91.2 | 14.3 | 4.0 | 94.4 | 15.0 |
| Mean ± SE | 17.4 ± 2.2 | 79.0 ± 4.6 | 83.4 ± 3.3 | 13.9 ± 1.4 | 4.6 ± 0.8 | 92.0 ± 2.4 | 37.4 ± 7.6 |

Reference strains used in crosses are Nasr'allah (W), Riverside (R), Seychelles-81 (S). Data given are percentages of unhatched eggs (Uh) per cross. Number of eggs for each cross $140 < N < 269$, except (a) = 116, (b) = 130, (c) = 131.

incompatibilities are unidirectional and result in reduced egg eclosion when S or R type males are crossed with W-type females. Incompatibility decreases in intensity when males become older and is substantially reduced by tetracycline treatment. Tetracycline treatment of R (Riverside) and S-type (Hawaii) strains restored bidirectional compatibility (O'NEILL and KARR 1990). Given that S and R are bidirectionally incompatible, and that both are incompatible with W strains, it seems probable that S and R either possess independent factors producing these effects, or that different secondary factors are producing the two systems of incompatibility.

Wolbachia-like microorganisms (WLOs) may be directly responsible for these incompatibilities. WLOs have been found in R type strains (LOUIS and NIGRO 1989; BINNINGTON and HOFFMANN 1990). WLOs observed in the testes and ovaries of S flies are morpho-

logically indistinguishable from those found in gonads of R flies (C. LOUIS, personal communication). BREUWER and WERREN (1990) found that bidirectional incompatibility between two closely related wasp species is probably caused by two different types of microorganisms. Thus all R-type strains may contain the same type of WLOs, whereas a different type may be present in S strains. Alternatively, if the symbionts are identical, R and S-type strains may genetically differ for their response to infection.

Consequences of infection: In *C. pipiens*, parthenogenetic females can be produced as a result of incompatibility (JOST 1970). No virgin females were observed to produce offspring, and a normal sex ratio occurs among progeny of incompatible crosses. However in the progeny of males from crosses between S-type females and W- or R-type males, we observed a significant excess of female eggs (data not shown).

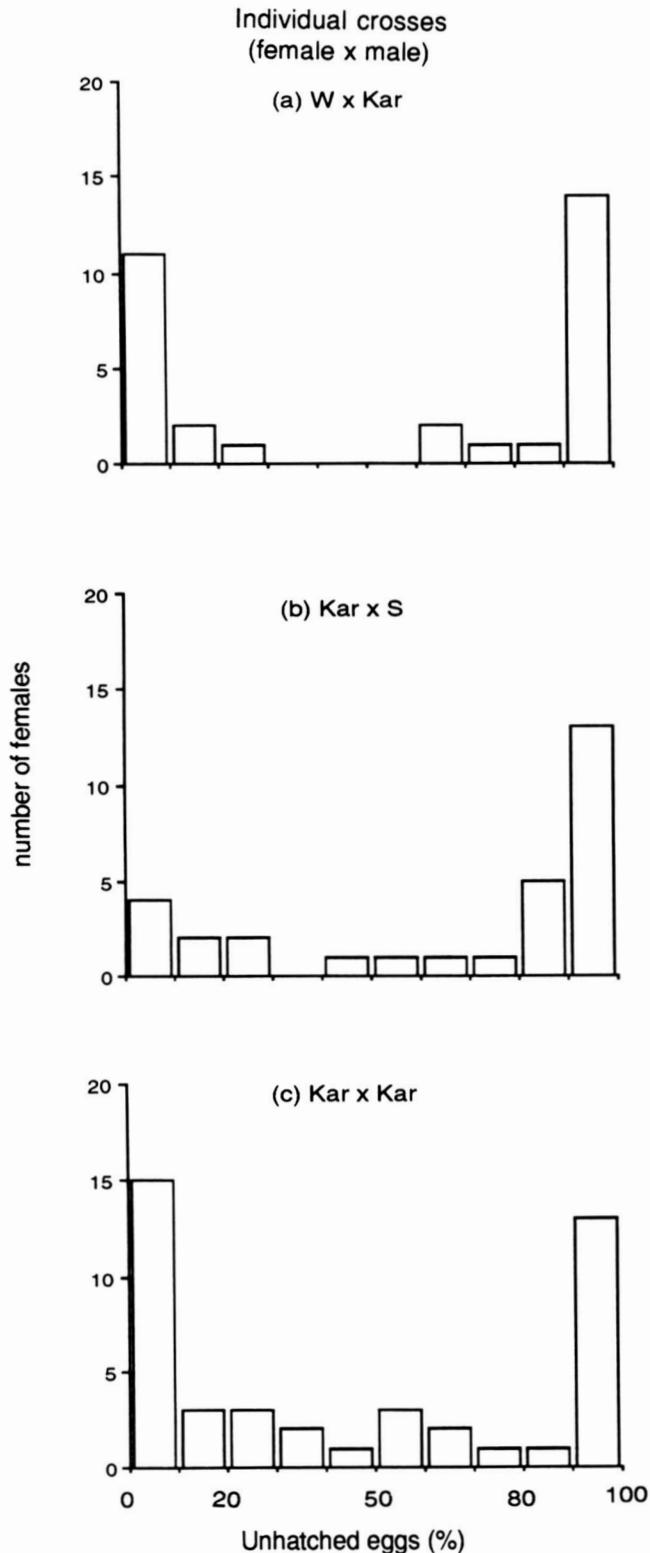


FIGURE 2.—Distribution of the percentage of unatched eggs laid by each female in the following individual crosses: (a) Nasr'allah female × Karaka male, (b) Karaka female × Seychelles-81 male, (c) Karaka female × Karaka male. Kar = Karaka, S = Seychelles-81, W = Nasr'allah.

WLOs have been detected in mature ovocytes and spermatocytes, but not in spermatozoons, of infected

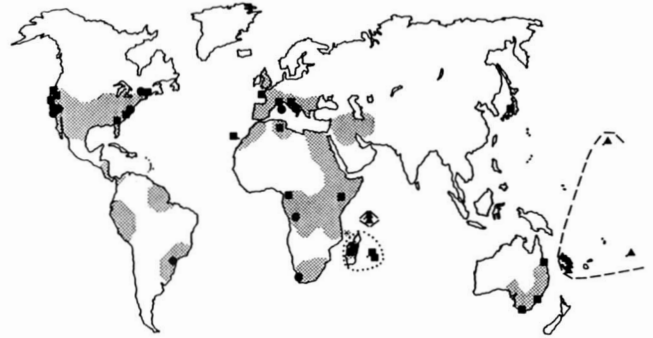


FIGURE 3.—Geographical distribution of incompatibility types and mtDNA types in *D. simulans*. Data for mtDNA types are from Baba-Aïssa *et al.* (1988): (□) regions in which SiII type has been found; (dotted area,) region with mixed SiII and SiIII types; (dashed area, - - - -) region where SiI type was found. Locations for incompatibility types are given in Table 1. (■) type W, (●) type R, (▲) type S.

flies (BINNINGTON and HOFFMANN 1990). Microorganisms are probably maternally transmitted through incorporation into the pole cells of fertilized eggs. Abnormal DNA structures were observed during the first mitotic cleavage in infected embryos, and are probably the cause of development failure (O'NEILL and KARR 1990). Taken together, these data suggest that incompatibility in *D. simulans* may be based upon on paternal chromosome loss, as observed in fertilized incompatible eggs of the parasite wasp *Nasonia vitripennis* (BREEUWER and WERREN 1990).

Transmission of incompatibility: As previously shown for W and R types, S-type incompatibility is partially maternally inherited. We also found an important paternally transmitted hereditary effect which substantially alters the strength of the S-type infection: crosses of S-cytype females with W males produced flies with an increasingly weak S phenotype as the proportion of W factors increased (Tables 4 and 5). Incompatibility level is correlated with the number of WLOs observed in the testes of infected males (BINNINGTON and HOFFMANN 1990); symbiont number may thus decrease in S × W progeny. Biparental chromosomal host control of the symbiont population has been proposed in weevils to explain stable differences in the number of symbionts between isofemale lines (NARDON and GRENIER 1988). W flies could thus express characters that change the strength of the S infection in both sexes.

S males were never observed to change the cytype of the offspring of W females. However, HOFFMANN and TURELLI (1988) report one case of an R male transmitting WLOs to the descendants of a W female. This result could imply that the number of symbionts in progeny is determined by both parental cytypes. We are currently studying treated and untreated S-type lines in order to evaluate both nuclear and cytoplasmic effects on the inheritance of incompatibility.

Unidirectional incompatibility system variation:

Differences in the number of symbionts in individual flies might explain the variability observed within and between S-type strains (Figures 1 and 2; Table 6C). However, intrastain differences might also produce variable levels of incompatibility in individual crosses. In general, there was a correlation for incompatibility levels produced by males and females of a given S-type strain: the more males were compatible with W females, the more females of this S-type strain were incompatible with S reference males.

Do W strains result from a noninfected ancestor or from flies naturally cured in the wild? Our data suggest that the presence of W (symbiont-free) flies and S (infected) flies in the same strain (*e.g.*, Karaka) might be partly due to a curing effect of W nuclear factors. Some Californian strains show a stable R/W polymorphism which seems to be maintained by a curing effect rather than by migration (HOFFMANN, TURELLI and HARSHMAN 1990). Maintenance of the R/W and S/W polymorphisms could be explained by an equilibrium between unidirectional transfer of nuclear material from the noninfected strain into the infected one and the subsequent spread of the infected cytotype, assuming that there was no fitness difference between the two cytotypes (CASPARI and WATSON 1959). Maintenance of the R/W polymorphism may be enhanced by higher fitness (progeny production) of the W strain and lower incompatibility in the wild than in the laboratory (HOFFMAN, TURELLI and HARSHMAN 1990).

mtDNA and unidirectional incompatibility: R and S infections are, respectively, associated with the SiII and SiI mitochondrial types. Any model of the evolution of incompatibility in *D. simulans* must therefore allow for the fact that mtDNA types reveal a high level of geographical differentiation between S-type and R-type populations. It should also be noted that incompatibility types and mtDNA types, both of which are maternally inherited, represent two cosegregating markers of cytoplasmic lineages which appear to be associated with some kind of "hitchhiking" dynamic, where the increase in the frequency of one incompatibility type apparently promoted the spread of the cytoplasmic lineage it happened to infect.

A unique ancestral infection event may have taken place prior to the differentiation of the SiI and SiII mitochondrial types. Divergence between symbionts could then have occurred at the same time as that between mtDNA types. Bidirectional incompatibility would have created an isolation between the two mtDNA types. On the other hand, R-type isofemale lines collected in distant locations carry the same SiII mtDNA "B subtype" (HALE and HOFFMANN 1990). Type R and type S may thus each result from a different infection event, occurring after the mito-

chondrial types SiI and SiII had separated.

The theoretical model of CASPARI and WATSON (1959) predicts that infected cytoplasm should either be fixed or eliminated in isolated populations. This simple model could explain monomorphic island populations; migration and more complex environmental patterns could maintain patches of R-W polymorphism in mainland populations (HOFFMAN, TURELLI and HARSHMAN 1990). A final possibility is that infection of *D. simulans* by WLOs may occur frequently. The geographical distribution of S and R incompatibility types would thus reflect the distribution of two different symbiont types or a functional link between mitochondrial type and microorganism identity.

Existence of an S/W polymorphism within S-type strains might also be linked with the SiI mtDNA polymorphism. Among SiI mtDNA types, five subtypes were found (BABA-AÏSSA *et al.* 1988). SiIb, which is the most frequent subtype, is probably associated with the S infection. An extensive study of mtDNA in S type isofemale lines will be necessary to determine if different SiI subtypes are systematically linked with variations of incompatibility level between and within S type strains.

Evolutionary implications: WLOs may be playing a role in an incipient speciation process in *D. simulans*. Whether the isolation between strains carrying different microorganisms is effective in natural conditions is not known. Data on allozyme polymorphisms show that genetic distances between *D. simulans* populations are low and weakly correlated with the geographical distances (CHODARY and SINGH 1987), probably due to a recent and worldwide expansion of this species through human transport. Nevertheless, populations from the Seychelles archipelago, characterized with allozyme data, appear significantly isolated from all other studied populations (SiII and SiIII) whatever the geographical distances (HYTTIA *et al.* 1985; CHODARY and SINGH 1987). Further studies of the S incompatibility system should bring valuable results in the fields of coevolution and speciation, as well as increasing our understanding of host genetic responses to symbiont infection.

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