Cytoplasmic Incompatibility in Australian Populations of *Drosophila melanogaster*

Ary A. Hoffmann, David J. Clancy and Eve Merton

Department of Genetics and Human Variation, La Trobe University, Bundoora, Victoria 3083, Australia

Manuscript received August 5, 1993
Accepted for publication November 13, 1993

ABSTRACT

In *Drosophila melanogaster*, weak incompatibility in crosses between infected and uninfected strains is associated with a Wolbachia microorganism. Crosses between infected males and uninfected females show a reduction (15–30%) in egg hatch. Progeny tests indicated that the infection is widespread in Australian *D. melanogaster* populations and that populations are polymorphic for the presence of the infection. The infection status of 266 lines from 12 populations along the eastern coast of Australia was determined by 4′,6-diamidino-2-phenylindole (DAPI) staining of embryos. All populations contained both infected and uninfected flies. Infection frequencies varied between populations but there was no discernible geographical pattern. Laboratory experiments indicated that the infection was not associated with a reduction in fecundity as in *Drosophila simulans*. Incompatibility levels could not be increased by laboratory selection on isofemale lines. Factors contributing to the persistence of the infection in *D. melanogaster* populations are discussed.

CYTOPLASMIC incompatibility in insects has often been associated with the presence of a maternally inherited microorganism of the genus *Wolbachia* (YEN and BARR 1974; HSIAO and HSIAO 1985; LOUIS and NIGRO 1989; BINNINGTON and HOFFMANN 1989). Incompatibility occurs when infected males are mated with uninfected females, or when matings take place between individuals with different strains of the infection. Molecular probes for Wolbachia indicate that the infection is widespread in insects (O'NEILL *et al.* 1992; ROUSSET *et al.* 1992; BREEUWER *et al.* 1992; STOUTHAMER *et al.* 1993).

Most research has focused on incompatibility systems where there is a drastic reduction of progeny numbers when infected males are crossed with uninfected females. Examples include the incompatibility systems of flour beetles (STEVENS and WADE 1990), parasitic wasps (BREEUWER and WERREN 1990), planthoppers (NODA 1987), alfalfa weevils (LEU *et al.* 1989) and mosquitoes (YEN and BARR 1974). Drastic effects are also found in the incompatibility system of *Drosophila simulans*, where incompatible crosses involving young infected males produce less than 5% of the progeny produced by compatible crosses (HOFFMANN *et al.* 1986).

In contrast, Wolbachia infections in other species may cause only weak incompatibility. HOFFMANN (1988) described an incompatibility system in *Drosophila melanogaster*, a sibling species of *D. simulans*, where incompatible crosses produce 70–85% of the progeny numbers produced by compatible crosses. This weak incompatibility was detected in crosses between stocks from Townsville (19°S) and Melbourne (38°S) on the east coast of Australia. The *D. melanogaster* system is associated with an infection similar to that causing incompatibility in *D. simulans* and other species as determined by sequence homology in the 16S rRNA gene (HOLDEN *et al.* 1993). Incompatibility levels in *D. melanogaster* are probably lower than in *D. simulans* because of differences in nuclear genetic background rather than in the microorganism. When the *D. simulans* infection is introduced by microinjection into a *D. melanogaster* background, a low level of incompatibility results (BOYLE *et al.* 1993). This level is similar to that found in crosses between naturally infected strains (HOFFMANN 1988), suggesting that the *D. melanogaster* nuclear background reduces incompatibility.

The population dynamics of incompatibility in *D. simulans* have been studied extensively (*e.g.*, HOFFMANN *et al.* 1990; TURELLI and HOFFMANN 1991; TURELLI *et al.* 1992). As a result, many of the processes influencing infection frequencies in natural populations of *D. simulans* are now known. Similar studies on *D. melanogaster* would allow the evolution of these different incompatibility systems to be compared. Unfortunately, population studies on *D. melanogaster* have been difficult because low levels of incompatibility mean that large numbers of replicate crosses are needed to ascertain the infection status of a line by progeny testing.

In this report, we investigate the distribution of the incompatibility infection within and among Australian populations of *D. melanogaster*. We have used progeny testing to examine the infection status of lines and polymorphism within populations. In addition, we describe and verify a technique for determining infection status involving staining of embryos with the DNA-specific 4′,6-diamidino-2-phenylindole (DAPI) dye, which has previously been used in *D. simulans* by O'NEILL and KARR

TABLE 1

Origin of *D. melanogaster* stocks used in crosses

Origin	Collection date	Description
Townsville (T1)	10/86	Massbred from 30 isofemale lines
Townsville (T2)	5/92	Massbred from 25 isofemale lines
Melbourne (M1)	10/86	Massbred from 30 isofemale lines
Melbourne (M2)	5/92	Massbred from 25 isofemale lines
Coffs Harbour	12/86	Massbred from 25 isofemale lines
Coffs Harbour	6/88	31 isofemale lines
Coffs Harbour	2/91	50 isofemale lines
Lakes Entrance	3/89	8 isofemale lines
Carnarvon	?/91	Massbred from field larvae
Perth	?/91	Massbred from 100 field adults
Dunsborough	?/91	Massbred from 30–40 field adults
Cairns	11/89	Massbred from 20 field adults

(1990). This technique is used to characterize infection frequencies in populations along the eastern coast of Australia.

This study has two subsidiary aims. First, we examine the potential of incompatibility levels to change in *D. melanogaster* populations by selecting for an increase in incompatibility. Such an increase might be associated with cytoplasmic factors (including the Wolbachia infection) as well as nuclear genes. Second, we test for fitness differences between infected and uninfected flies. Previous experiments have demonstrated that infected *D. simulans* lay fewer eggs than uninfected flies (HOFFMANN *et al.* 1990), and we test for a similar effect in *D. melanogaster*. We use this information to discuss the distribution of incompatibility types in Australian *D. melanogaster* populations.

MATERIALS AND METHODS

Stocks: A number of Australian *D. melanogaster* stocks were used for progeny testing and the laboratory experiments (Table 1). Incompatibility in this species was initially detected in crosses between stocks originating from Townsville and Melbourne. Mass-bred stocks from these locations described in HOFFMANN (1988) were used in some of the experiments. Freshly collected Melbourne and Townsville stocks were obtained for later experiments. Crosses were also carried out with a mass-bred stock from Coffs Harbour in New South Wales and with three mass-bred stocks from Western Australia (Carnarvon, Perth and Dunsborough). To test for polymorphism within populations, isofemale lines were set up from individual field-collected females. Isofemale lines from Lakes Entrance (Victoria) and Coffs Harbour were characterized by progeny testing.

To examine infection frequencies in more detail, 266 isofemale lines were set up from females collected in February from 12 locations along the east coast of Australia (Figure 1). These lines were characterized for infection status by DAPI staining.

All stocks were maintained at 19–24° as discrete generations on a sucrose-agar-dead yeast medium by mass transfer of at least 80 adults per generation. Some stocks were cured of the infection causing incompatibility by exposing larvae to laboratory medium with 0.03% tetracycline following HOFFMANN (1988).

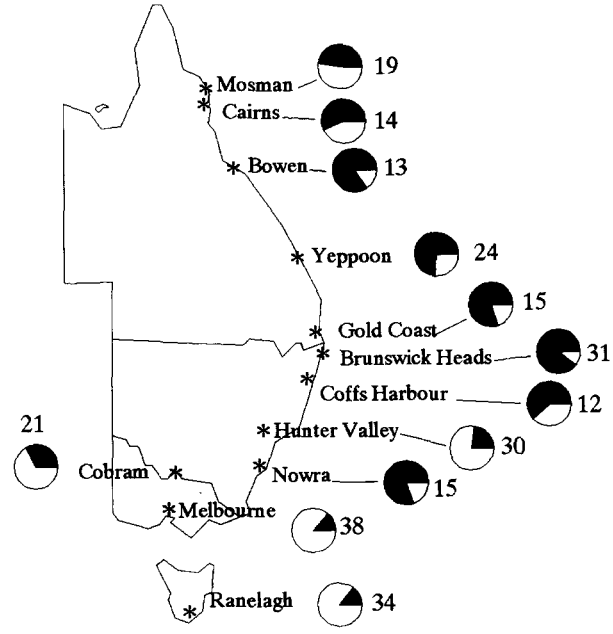

FIGURE 1.—Map of eastern Australia showing the frequency of infection at different sites detected by DAPI technique (filled area of pie graphs) and sample sizes (numbers after graphs).

Progeny testing: The infection status of some strains was scored by mating females to an infected stock, and by mating males to an uninfected stock. If a strain is infected, incompatibility should be evident when males are mated with uninfected females. If a strain is uninfected, incompatibility is found in crosses to infected males. Crosses within strains were often carried out to ensure that any apparent incompatibility was not a consequence of deleterious nuclear genes segregating in a strain.

Incompatibility in *D. melanogaster* is manifested as a reduction in hatch rate (HOFFMANN 1988). This was scored in two ways. In the first approach, 5 or 10 eggs were placed in a vial with medium. Incompatibility was determined from the number of emerging adults. At least 10 replicate vials were set up for a cross, and eggs for each vial were obtained from a different female. In the second approach, individual females were allowed to lay eggs on a spoon filled with black currant medium as described in HOFFMANN *et al.* (1990). The eggs were left for a day at 25° to ensure that larvae from viable eggs hatched. Hatch rates were determined only for spoons with at least 10 eggs.

Detection of infection by DAPI: We used a modification of the technique in O'NEILL and KARR (1990). Approximately 80 flies (less than 5 days old) were fed on yeasted medium in bottles for 1–3 days at 25°. Flies were then tipped into empty bottles and allowed to lay on yeasted black currant juice medium on watch glasses covering the bottle mouths. Flies were allowed to lay for about 1 hr at 25° in darkness. Eggs were washed from the watch glass into a small beaker using a solution of 0.4% NaCl and 0.03% Triton X-100, transferred to a 10-ml Bunzl tube and washed until the solution was clear. The wash solution was removed and replaced with a solution of 15 g/liter hypochlorite (for dechorionation). Tubes were capped and shaken gently for about 90 sec before replacing hypochlorite with wash solution and washing twice. Using a large bore micropipet tip, embryos were transferred to 10-ml conical bottomed glass tubes and 2 ml each of heptane and methanol

TABLE 2

Crosses between Coffs Harbour flies and reference (M1, T1) strains

Cross	Mean no. of adults from 5 eggs	SD	*N*
a. M1 ♀ × Coffs H ♂	2.79	1.72	19
b. M1 × M1	4.47	0.62	17
c. Coffs H × Coffs H	4.33	0.42	15
d. Coffs H ♀ × T1 ♂	4.68	0.58	19
e. M1 ♀ × T1 ♂	3.18	1.58	22

were added for vitelline removal. Tubes were capped and shaken vigorously for 60 sec. Liquid was removed and embryos washed twice with methanol then once with PBST (phosphate-buffered saline (PBS) with 0.1% Triton X-100). Eggs were stained for 12 min in PBS containing 1 μg/ml DAPI, washed in PBS, mounted in 80/20 glycerol/PBS and viewed at 600× using a Zeiss epifluorescent microscope. Wolbachia are clearly visible surrounding nuclei and in the cortex of the embryo.

Fecundity: In *D. simulans*, the only laboratory fitness trait affected by the Wolbachia is fecundity (HOFFMANN *et al.* 1990). We therefore investigated the effect of the infection on this fitness component in *D. melanogaster* by comparing fecundity in an infected stock and an uninfected stock derived from it by exposure to tetracycline. These stocks should have the same nuclear background.

Mass-bred Cairns flies from a stock (C2) collected in 1989 (Table 1) were used in this experiment. This stock is known to be infected on the basis of DAPI staining (see below). Larvae from the stock were grown on medium with tetracycline for a generation. This cured stock was then grown for a generation on medium without tetracycline. Virgin females from the cured stock and the original Cairns stock were collected and aged at 25° in vials containing yeasted medium for 3 days. The females were then set up at 25° with 3-day-old virgin M2 (uninfected Melbourne) males as pairs in vials containing spoons with molasses medium lightly brushed with yeast suspension. Females were allowed to lay for 22 hr, when flies were removed and eggs were counted.

RESULTS

Progeny testing of Coffs Harbour stocks: Incompatibility in the 1986 Coffs Harbour stock was examined by counting the number of adults to emerge from groups of five eggs. The number of emerging flies were compared by non-parametric Mann-Whitney *U* tests because emergents showed a skewed distribution. The results (Table 2) indicate that fewer adults emerged from the cross between Coffs Harbour males and M1 females (c) than from the M1 × M1 cross (b) suggesting that Coffs Harbour is infected. This was confirmed by the absence of incompatibility in the cross between Coffs Harbour females and Townsville males (d), which did not differ significantly from the cross between Coffs Harbour males and females (c). As expected, T1 males are infected and cause incompatibility when mated to uninfected females from the Melbourne stock (e).

We next looked for polymorphism within this population by testing isofemale lines set up with females collected from Coffs Harbour in 1988. Males from each line were crossed to uninfected (M1) females, and 4 groups of 10 eggs were placed into vials to characterize incompatibility levels. From the 31 lines tested, two with the lowest hatch rates (both 47%) and two with the highest rates (95 and 97%) were selected for further characterization. Males from these lines were mated to M1 females and 20–25 groups of 10 eggs were set up for incompatibility testing. The two "low" lines both had mean hatch rates of 57%. One of the high strains had a hatch rate of 88% but the other only had a 56% hatch rate, indicating a low level of repeatability between experiments.

TABLE 3

Crosses involving selected lines from Coffs Harbour (C8, C21) and an uninfected stock (M1)

Cross	Mean no. of adults from 10 eggs	SD	*N*
a. C8 × C8	8.90	1.51	22
b. C21 × C21	7.95	2.66	20
c. M1 ♀ × C8 ♂	5.08	2.87	13
d. M1 ♀ × C21 ♂	9.58	0.67	12
e. M1 × M1	8.92	1.38	13

We set up crosses within one of the low lines (C8) and the consistent high line (C21) to check for inbreeding effects, and we also crossed these lines to M1 females. Incompatibility was scored as the number of progeny produced by groups of 10 eggs. The results (Table 3) confirm those of the previous experiment. Egg hatchability was reduced significantly (Mann-Whitney *U* test, $P < 0.001$) when C8 males were crossed to M1 females (cross c) compared to the cross among Melbourne males and females (e). In contrast, progeny numbers were not reduced when C21 males were crossed to Melbourne females (d), suggesting that this line was uninfected. Although crosses within the C21 and C8 lines produced high hatch rates, there was some suggestion of inbreeding in the lines because adult numbers were lower than those obtained from the cross between M1 females and C21 males.

Because of the possibility of inbreeding, further comparisons were made between pairs of reciprocal crosses (Table 4). In the first pair of crosses (pair a), flies from the C8 line were mated reciprocally with Townsville flies. These crosses did not differ significantly indicating that C8 behaved like the infected Townsville line. In reciprocal crosses between C8 and C21 flies (b), adult numbers were significantly lower when C8 males were crossed to C21 females, demonstrating incompatibility between these lines originating from the same natural population.

Crosses were also made using a C8 line treated with tetracycline for a generation to cure the putative infection (Table 4). When the treated C8 line was crossed reciprocally to T1 flies, progeny numbers differed significantly between the crosses (cross pair c). The treated

TABLE 4

Reciprocal crosses involving tetracycline treated (tet) and untreated C8 flies and reference stocks

Reciprocal cross pair	Mean no. of adults from 10 eggs	SD	N	P
a. C8 ♀ × T1 ♂	9.10	1.48	20	NS
T1 ♀ × C8 ♂	9.10	1.21	20	
b. C21 ♀ × C8 ♂	6.85	2.16	20	<0.001
C8 ♀ × C21 ♂	9.74	0.45	19	
c. C8(tet) ♀ × T1 ♂	7.60	1.57	20	<0.05
T1 ♀ × C8(tet) ♂	8.55	1.61	20	
d. C8(tet) ♀ × C8 ♂	5.75	2.30	20	<0.001
C8 ♀ × C8(tet) ♂	8.65	1.53	20	
e. C8(tet) ♀ × M1 ♂	8.53	1.43	19	NS
M1 ♀ × C8(tet) ♂	9.00	1.38	21	

Probabilities are for Mann-Whitney tests comparing each pair of crosses.

TABLE 5

Crosses between stocks from Western Australia and reference stocks

Cross	Mean proportion of eggs hatched	SD	P
a. Dunsborough × Dunsborough	0.98	0.02	<0.001
Dunsborough ♀ × T1 ♂	0.87	0.06	
b. Carnarvon × Carnarvon	0.97	0.03	NS
Carnavon ♀ × T1 ♂	0.94	0.05	
c. Perth × Perth	0.98	0.02	NS
Perth ♀ × T1 ♂	0.93	0.15	
d1. M1 × M1	0.98	0.02	
d2. M1 ♀ × T1 ♂	0.78	0.04	<0.001
d3. M1 ♀ × Dunsborough ♂	0.96	0.04	NS
d4. M1 ♀ × Carnarvon ♂	0.90	0.04	<0.01
d5. M1 ♀ × Perth ♂	0.79	0.08	<0.001

Probabilities are for Mann-Whitney tests comparing pairs of crosses (a, b, c) or comparing each of four crosses (d2, d3, d4, d5) to M1 × M1 (d1). Means are based on 10 replicates.

C8 line behaved like an uninfected stock, although the difference between the crosses was smaller than in the other comparisons. Following treatment with tetracycline, the C8 line became incompatible with flies from the untreated C8 line (d), and it was incompatible with Melbourne flies (e).

These experiments provide the first evidence for polymorphism within a *D. melanogaster* population. As in *D. simulans* (HOFFMANN and TURELLI 1988), both infected and uninfected flies may occur in the same natural population. The C8 line behaves the same as the mass-bred Townsville stock described in HOFFMANN (1988). A further series of crosses with Coffs Harbour isofemale lines collected in 1989 (not presented) confirmed the existence of infected and uninfected lines in this population as determined by progeny tests.

Progeny testing of other Australian populations: We tested eight isofemale lines collected from Lakes Entrance in 1989. Five males from each line were crossed to Melbourne females and laid eggs on spoons. Incompatibility was determined from the number of eggs that failed to hatch. All except two of the lines appeared to be compatible because they produced hatch rates of 90% or greater, based on samples of 100 eggs or more. The two aberrant lines were retested by crossing 15–20 males to Melbourne females and comparing hatch rates with those from the reciprocal crosses. Incompatibility was not detected in this second set of crosses, although it was evident in control crosses between Melbourne females and Townsville males. The eight lines from Lakes Entrance therefore all behaved as if they were uninfected.

Progeny tests were also carried out with three mass-bred stocks from Western Australia. In this case, incompatibility was scored directly by examining hatch rates of eggs laid on medium in spoons. Crosses between T1 males and females from the populations were compared to crosses within the stocks (Table 5). One population (Dunsborough) behaved like an uninfected stock in these crosses (a). The other two populations (Carnarvon, Perth) behaved as if they were infected (b, c). This was confirmed by crosses between males from the stocks and M1 females. Mann-Whitney tests indicated significant differences between the M1 × M1 cross and those involving Carnarvon (d4) and Perth (d5) males. In contrast, Dunsborough males behaved like flies from an uninfected stock (d3). Both infected and uninfected stocks could therefore be isolated from Western Australia *D. melanogaster*.

Characterization of lines by DAPI: We initially tested the DAPI technique on lines previously characterized by progeny testing. As expected, the T1 line tested positive in the DAPI assay, whereas M1 tested negative. The Townsville (T2) and Melbourne (M2) lines collected in 1992 (Table 1) also tested positive and negative, respectively. In addition, the Coffs Harbour lines C8 and C21 tested positive and negative, respectively, consistent with their behavior in progeny tests. The Dunsborough line from Western Australia was DAPI-negative, whereas positive results were obtained with the Carnarvon and Perth lines, as well as with a mass-bred Cairns stock collected in 1989 (Table 1). DAPI therefore accurately identified six infected lines and four uninfected lines previously characterized by progeny testing, suggesting its usefulness in population studies of infection frequencies. We used the DAPI assay to determine infection levels in populations from the east coast of Australia. Female *D. melanogaster* were collected from 12 locations extending from northern Queensland to Tasmania (Figure 1) in 1993. The 266 isofemale lines from these locations were characterized for infection status 2–12 generations after lines had been established in the laboratory.

All 12 populations were polymorphic and infection frequencies ranged from 18 to 85%. Differences between populations were highly significant by a contingency test ($G = 83.3$, d.f. = 11, $P < 0.001$). There were no consistent geographic patterns in infection frequencies (Figure 2). Some adjacent populations differed sig-

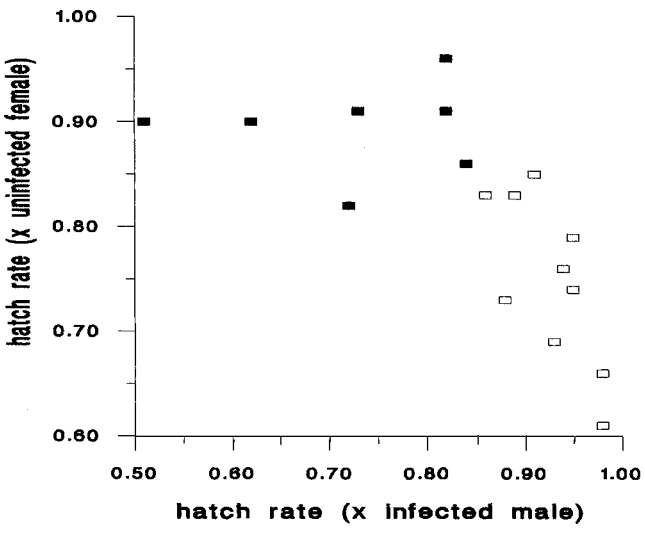

FIGURE 2.—Mean hatch rates of crosses between uninfected M2 females and males from isofemale lines plotted against hatch rates of crosses between infected T2 males and females from the lines. Open squares indicate DAPI-positive strains and filled squares indicate DAPI-negative strains.

nificantly. For instance, frequencies in the Nowra and Hunter Valley collections differed ($G = 13.7$, d.f. = 1, $P < 0.001$), as did frequencies in the Mosman/Cairns and Bowen collections ($G = 4.7$, d.f. = 1, $P < 0.05$). In contrast, infection frequencies in southern Queensland and northern New South Wales were similar, as were those from Victoria and Tasmania.

To further compare the DAPI results with those from progeny testing, we carried out crosses with 7 DAPI-negative lines and 10 DAPI-positive lines. We crossed 8–10 males from each line to M2 females, and 8–10 females to T2 males. Hatch rates were scored directly from eggs laid on spoons. The results of these tests (Figure 2) indicate that the DAPI-negative and DAPI-positive groups separated as expected, particularly on the basis of crosses to infected males. However, three of the DAPI-positive lines showed rather high hatch rates when crossed to uninfected females. Further crosses were carried out with these three lines by crossing them reciprocally to the M2 stock. In each pairwise comparison, crosses between males of the line and M2 females produced significantly lower hatch rates than the reciprocal cross, confirming that these lines behaved as if they were infected.

Segregation in Coffs Harbour: We attempted to increase levels of incompatibility in crosses between infected and uninfected males by repeatedly selecting among isofemale lines. We started with 50 isofemale lines set up from females collected in Coffs Harbour in 1991. Two males from each of these lines were crossed to M1 females when they were 1–2 days old. Four lines with the highest overall incompatibility levels were chosen for further selection. Five isofemale lines were established for each of these lines and two males from each line were crossed to M1 females. The five lines with highest incompatibility levels were selected. Five isofemale lines were again set up from these lines, and the selection procedure was repeated a third time. This led to 10 isofemale lines with apparently high levels of incompatibility from three rounds of selection.

These lines were characterized in crosses to M1 females. For each line, 10–15 males were crossed to M1 females, and incompatibility levels compared to those produced from control crosses between M1 females and T1 males. Only two lines (E8, E9) differed significantly ($P < 0.05$) in hatchability from the control crosses. These lines had hatch rates of 36 and 48%, whereas the others had hatch rates <60%.

Altered levels of incompatibility in the E8 and E9 lines may be due to nuclear genes influencing incompatibility levels, cytoplasmic factors including variation in the Wolbachia infection, or they may be an artifact of deleterious genes in the lines that influence male fertility. To distinguish between these possibilities, lines were crossed reciprocally to the T1 infected stock so that F_1s would have the cytoplasmic background of the lines or T1 stock. Fifteen F_1 males from each reciprocal cross were mated to M1 females to compare incompatibility levels. The same number of crosses were set up between T1 males and M1 females. The hatch rates of all crosses with the F_1 males was in the range 82–85%, while mean hatch rates in the control (M1 ♀ × T1 ♂) cross was 84%. Because none of the reciprocal F_1s differed significantly, cytoplasmic factors are not responsible for the low hatch rates in the parental E8 and E9 lines. In addition, the similar hatch rates in the control cross and the F_1 crosses suggest that the low hatch rates of the E8 and E9 lines are an artifact of deleterious nuclear genes unrelated to incompatibility. This was confirmed by the persistence of low hatch rates in crosses with E8 and E9 stocks that had been treated with tetracycline. We were therefore unsuccessful in changing incompatibility levels by selecting on Coffs Harbour lines.

Fecundity: This experiment tested for deleterious effects associated with the infection by comparing the fecundity of an infected strain with that of an uninfected strain derived from it by treating the strain with antibiotics. The mean number of eggs produced by the infected females over 22 hr was 81.0 (SD = 15.3, $n = 40$), while the mean for the uninfected females was 82.6 (SD = 82.6, $n = 38$). Means were compared using a one-tailed t-test because infected flies were expected to have a lower fecundity than uninfected flies. The difference between these means was not significant ($t = -0.52$, d.f. = 76, $P = 0.31$), so there was no evidence for a deleterious effect associated with the infection.

DISCUSSION

The population survey suggests that DAPI staining of embryos is a useful way of determining the infection status of *D. melanogaster* lines because the results obtained with this technique agree with those from prog-

eny tests. DAPI testing has also been compared to screening for infection using Wolbachia-specific primers amplified by polymerase chain reaction (PCR) (M. TURELLI and A. A. HOFFMANN, unpublished). Of the 60 *D. melanogaster* strains tested so far, there is agreement in 56 cases (32 positive lines, 24 negative lines). The four inconsistent lines were originally negative by the DAPI assay and positive by PCR. However, they were positive in a later DAPI assay, suggesting that a low level of infection detectable by PCR may occasionally not be detected by staining embryos. DAPI staining of sperm cysts may represent an alternative approach to embryo staining as recently demonstrated in *D. simulans* (BRESSAC and ROUSSET 1993).

The progeny tests and DAPI data indicate that Australian populations of *D. melanogaster* are polymorphic for the Wolbachia infection. Polymorphism may also occur in other *D. melanogaster* populations, and has recently been detected using Wolbachia-specific primers in a California population of *D. melanogaster* (M. TURELLI, unpublished). This geographic pattern of infection can be contrasted to the situation in *D. simulans* where the dynamics of the incompatibility system are understood (HOFFMANN *et al.* 1990; TURELLI and HOFFMANN 1991; TURELLI *et al.* 1992). In California *D. simulans* populations, the infection was initially confined to an area south of the mountain ranges that separate the Central Valley from the Los Angeles region. In this region, around 94% of the strains carry the infection. Some uninfected strains persist here because infected females occasionally produce uninfected progeny due to segregation. After 1986, the infection spread rapidly northward into the Central Valley area and along the western coast. Once the infection was detected in a population, it increased rapidly until more than 90% of flies were infected. *D. simulans* populations with intermediate infection frequencies were therefore in a transient state.

These changes in frequency have been successfully related to estimates of parameters influencing incompatibility in *D. simulans*. Following HOFFMANN *et al.* (1990), the change in frequency of the infection (p_t) in a population is given by

$$p_{(t+1)} = \frac{p_t(1-u)(1-s_f)}{1-s_f p_t - s_h p_t(1-p_t) - u s_h p_t^2(1-s_f)}, \qquad (1)$$

where u is the fraction of uninfected progeny produced by infected females, s_f is the fecundity deficit and s_h the incompatibility between infected and uninfected strains measured under field conditions. In *D. simulans*, u is in the range 0.01–0.06, s_f is around 0.0–0.1, and s_h is around 0.2–0.6 (HOFFMANN *et al.* 1990). These values lead to the expectation of a rapid increase of the infection once its frequency exceeds a low unstable point, and they also predict a stable infection frequency of around 0.94 as seen in populations from southern California.

Estimates for these parameters in *D. melanogaster* are likely to differ from those in *D. simulans*. Our results suggest that the infection does not decrease fecundity in *D. melanogaster*, unlike in *D. simulans* where decreases of 8–18% have been detected in similar laboratory experiments. Power tests indicate that a 5% difference in fecundity could have been detected in our experiment, so any fecundity deficit in *D. melanogaster* is small or absent. This may reflect a relatively lower density of Wolbachia in *D. melanogaster* compared to *D. simulans*. BOYLE *et al.* (1993) demonstrated that Wolbachia derived from *D. simulans* and injected into *D. melanogaster* occurred at a relatively lower density in its new host, although data on Wolbachia density have not yet been obtained for naturally occurring *D. melanogaster* infections.

Incompatibility levels in field *D. melanogaster* populations are likely to be lower than in *D. simulans*. In the laboratory, levels of incompatibility (percentage of eggs that do not hatch) in crosses with young *D. melanogaster* males are around 20–30% (HOFFMANN 1988), and this is much lower than in laboratory crosses with young *D. simulans* where incompatibility levels can exceed 95% (HOFFMANN *et al.* 1986). Levels of incompatibility are variable in *D. simulans* collected from the field, but lower than in the laboratory and range from 10% to 60%. This implies that *D. melanogaster* field levels are likely to be no greater than 25% and probably more likely to be around 10–20%.

How are these parameter estimates likely to influence infection frequencies in natural populations of *D. melanogaster*? If we set the fecundity deficit at 0, then following (1) the equilibrium frequencies of the infection are $p = 0$ and the roots of $s_h(1-u)p^2 - ps_h + u = 0$. If infected females do not produce uninfected progeny ($u = 0$), then the only equilibrium is $p = 1$, and the infection will become fixed. If segregation levels are similar to those in *D. simulans*, then persistent polymorphism is unlikely because of the lower levels of incompatibility in *D. melanogaster*. For instance, if we set u at 0.03 (on the basis of *D. simulans* data) and s_h at the level seen in laboratory *D. melanogaster* (0.25), then the unstable equilibrium is at $p = 0.18$ and the stable point is at $p = 0.84$. However, once s_h falls much below 0.20 there is no equilibrium, and the infection will always be lost. This suggests that fewer uninfected progeny need to be produced by *D. melanogaster* females than by *D. simulans* females if the infection is to persist under low levels of field incompatibility.

To determine whether infection frequencies are changing or at an equilibrium in populations of *D. melanogaster*, we need to carry out repeated sampling of the same populations. We also need field estimates of incompatibility levels and segregation rates in order to interpret any changes. These estimates should ideally be obtained for a number of *D. melanogaster* populations. Perhaps infection frequencies in *D. melanogaster* populations differ because they are affected by environmental

conditions such as heat and naturally occurring antibiotics that are known to reduce the expression of incompatibility in other species (HOFFMANN *et al.* 1990; STEVENS 1989). Such factors could influence segregation levels and deleterious fitness effects as well as incompatibility levels, causing infection dynamics to differ between populations.

How can the marked difference in host-parasite relationships between these closely related Drosophila species be explained? Once an infection is fixed in a population, host factors which reduce the production of uninfected progeny from infected females and lessen the deleterious fitness effects associated with infection should be selected (TURELLI 1994). The low level of incompatibility in *D. melanogaster*, absence of a deleterious fecundity effect and failure of our selection experiment to alter incompatibility levels could therefore reflect a history of selection on the nuclear genome of this species.

We thank L. PARTRIDGE, A. JAMES and K. GAULL for collecting strains and M. TURELLI for comments and useful discussions. This work was supported by the Australian Research Council via the Small Grants Scheme.

LITERATURE CITED

BINNINGTON, K. C., and A. A. HOFFMANN, 1989 Wolbachia-like organisms and cytoplasmic incompatibility in *Drosophila simulans*. J. Invert. Pathol. **54:** 344–352.

BOYLE, L., S. L. O'NEILL, H. ROBERTSON and T. L. KARR, 1993 Horizontal transfer of *Wolbachia pipientis* by microinjection of egg cytoplasm: infection levels and the expression of cytoplasmic incompatibility in Drosophila. Science **260:** 1796–1799.

BREEUWER, J. A. J., and J. H. WERREN, 1990 Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature **346:** 558–560.

BREEUWER, J. A. J., R. STOUTHAMER, S. M. BARNS, D. A. PELLETIER, W. G. WEISBURG and J. H. WERREN, 1992 Phylogeny of cytoplasmic incompatibility microorganisms in the parasitoid wasp genus *Nasonia* (Hymenoptera, Prteromalidae) based on 16S ribosomal sequences. Insect Mol. Biol. **1:** 25–36.

BRESSAC, C., and F. ROUSSET, 1993 The reproductive incompatibility system in *Drosophila simulans*: DAPI-staining analysis of the Wolbachia symbionts in sperm systs. J. Invert. Pathol. **61:** 226–230.

HOFFMANN, A. A., 1988 Partial cytoplasmic incompatibility between two Australian populations of *Drosophila melanogaster*. Entomol. Exp. Appl. **48:** 61–67.

HOFFMANN, A. A., and M. TURELLI, 1988 Unidirectional incompatibility in *Drosophila simulans*: geographic variation and fitness effects. Genetics **119:** 435–444.

HOFFMANN, A. A., M. TURELLI and G. M. SIMMONS, 1986 Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution **40:** 692–701.

HOFFMANN, A. A., M. TURELLI and L. G. HARSHMAN, 1990 Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. Genetics **126:** 933–948.

HOLDEN, P. R., P. JONES and J. F. Y. BROOKFIELD, 1993 Evidence for a *Wolbachia* symbiont in *Drosophila melanogaster*. Genet. Res. **62:** 23–29.

HSIAO, C., and T. H. HSIAO, 1985 Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil *Hypera postica*. J. Invert. Pathol. **45:** 244–246.

LEU, S.-J. C., J. K.-K. LI and T. H. HSIAO, 1989 Characterization of *Wolbachia postica*, the cause of reproductive incompatibility among alfalfa weevil strains. J. Invert. Pathol. **54:** 248–259.

LOUIS, C., and L. NIGRO, 1989 Ultrastructural evidence of Wolbachia Rickettsiales in *Drosophila simulans* and their relationship with unidirectional cross-incompatibility. J. Invert. Pathol. **54:** 34–44.

NODA, H., 1987 Further studies of cytoplasmic incompatibility in local populations of *Laodelphax striatellus* in Japan (Homoptera: Delphacidae). Appl. Entomol. Zool. **22:** 443–448.

O'NEILL, S. L., and T. L. KARR, 1990 Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature **348:** 178–180.

O'NEILL, S. L., R. GIORDANO, A. M. E. COLBERT, T. L. KARR and H. M. ROBERTSON, 1992 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. USA **89:** 2699–2702.

ROUSSET, F., D. VAUTRIN and M. SOLIGNAC, 1992 Molecular identification of Wolbachia, the agent of cytoplasmic incompatibility in *Drosphila simulans*, and variability in relation with host mitochondrial types. Proc. Roy. Soc. Lond. B **247:** 163–168.

STEVENS, L., 1989 Environmental factors affecting reproductive incompatibility in flour beetles, genus *Tribolium*. J. Invert. Pathol. **53:** 78–84.

STEVENS, L., and M. J. WADE, 1990 Cytoplasmically inherited reproductive incompatibility in Tribolium flour beetles: the rate of spread and the effect on population size. Genetics **124:** 367–373.

STOUTHAMER, R, J. A. L. BREEUWER, R. F. LUCK and J. H. WERREN, 1993 Molecular identification of microorganisms associated with parthenogenesis. Nature **361:** 66–68.

TURELLI, M., 1994 Evolution of incompatibility-inducing microbes and their hosts. Evolution (in press).

TURELLI, M., and A. A. HOFFMANN, 1991 Rapid spread of an inherited incompatibility factor in California Drosophila. Nature **353:** 440–442.

TURELLI, M., A. A. HOFFMANN and S. W. MCKECHNIE, 1992 Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. Genetics **132:** 713–723.

YEN, J. H., and A. R. BARR, 1974 Incompatibility in Culex pipiens, pp. 97–118 in *The Use of Genetics in Insect Control*, edited by R. PAL and M. J. WHITTEN. Elsevier, Amsterdam.

Communicating editor: C. C. LAURIE