

Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma*

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(Received 19 January 2000; revised 26 April 2000; accepted 30 April 2000)

SUMMARY

Endosymbiotic bacteria of the genus *Wolbachia* infect a number of invertebrate species in which they induce various alterations in host reproduction, mainly cytoplasmic incompatibility (CI). In contrast to most other maternally transmitted parasites, manipulation of host reproduction makes the spread of *Wolbachia* possible even if they induce a physiological cost on their hosts. Current studies have shown that fitness consequences of *Wolbachia* infection could range from positive (mutualist) to negative (parasitic) but, in most cases, *Wolbachia* do not have strong deleterious effects on host fitness and the status of association remains unclear. Here, we show that in the *Drosophila* parasitoid wasp *Leptopilina heterotoma*, *Wolbachia* infection has a negative impact on several host fitness traits of both sexes. Fecundity, adult survival and locomotor performance are significantly reduced, whereas circadian rhythm, development time and offspring sex-ratio are not affected. Although the cost of bacterial infection can be overcome by effects on host reproduction i.e. cytoplasmic incompatibility, it could influence the spread of the bacterium at the early stages of the invasion process. Clearly, results underline the wide spectrum of phenotypic effects of *Wolbachia* infection and, to our knowledge, *Wolbachia* infection of *L. heterotoma* appears to be one of the most virulent that has ever been observed in insects.

Key words: endosymbiosis, host–parasite evolution, virulence, *Wolbachia*, cytoplasmic incompatibility, fitness traits.

INTRODUCTION

Wolbachia are obligate intracellular bacteria (Rickettsiaceae) found in numerous species of invertebrates including insects, mites, crustaceans, and filarial nematodes (Werren, Zhang & Guo, 1995; O'Neill, Hoffmann & Werren, 1997; Bandi *et al.* 1998; Cook & Butcher, 1999). These vertically transmitted symbionts may alter host sexuality and reproduction in different ways which all enhance transmission of the bacteria in the host population. In parthenogenetic haplo–diploid wasp species, *Wolbachia* can revert reproduction from sexual (arrhenotokous) to asexual (thelytokous), with offspring consisting of infected females only (Stouthamer, Luck & Hamilton, 1990; Stouthamer, 1997). *Wolbachia* have also been shown to cause male killing in several insect species (Hurst *et al.* 1999). In isopod crustaceans, *Wolbachia* turn genetic males into functional females (feminization), and thus strongly interfere with sex determination (Rigaud, 1997). The last way *Wolbachia* alter their host reproduction is cytoplasmic incompatibility (CI)

which has been the first and the most commonly reported effect in a wide range of insects (Werren, 1997; Hoffmann & Turelli, 1997). *Wolbachia*-induced incompatibility results in embryonic mortality among the offspring of uninfected females when they have mated infected males, whereas the reciprocal cross is compatible (unidirectional incompatibility). CI also occurs between infected individuals harbouring different *Wolbachia* variants (bidirectional incompatibility) and then could contribute to reproductive isolation between populations and potentially lead to rapid speciation (Breeuwer & Werren, 1990).

Generally, maternally transmitted parasites can only be maintained in host populations if they do not reduce host fitness (Anderson & May, 1982; Smith & Dunn, 1991; Ebert & Herre, 1996). In contrast, manipulation of host reproduction allows *Wolbachia* to propagate even if they induce a physiological cost to their hosts, and this can prevent these associations evolving toward mutualism (Turelli, 1994; Werren, 1997; O'Neill *et al.* 1997). Evaluating this possible deleterious effect is thus of particular importance to clarify the status of host–*Wolbachia* associations on the mutualism–parasitism continuum (O'Neill, 1995). Moreover, it could help us to understand and predict the rate and extent of *Wolbachia* spread into host populations (Turelli, 1994; Vavre *et al.* 2000).

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Current studies have shown that in most cases *Wolbachia* have no effect on host fitness (Hoffmann, Clancy & Merton, 1994; Giordano, O'Neill & Robertson, 1995; Bourtzis *et al.* 1996; Poinsoot & Mercot, 1997; Hoffmann, Hercus & Dagher, 1998). In some cases, host fitness suffers slight reduction that depends on host species and genotype, on *Wolbachia* variants, and on fitness components considered (Hoffmann, Turelli & Harshman, 1990; Turelli & Hoffmann, 1995; Clancy & Hoffmann, 1997). The 'popcorn' variant has proved highly virulent and induces deleterious effects in a line of *D. melanogaster*, but its effect on host reproduction remains unknown (Min & Benzer, 1997). In contrast, enhanced reproductive success of infected individuals has been reported in some species (Wade & Chang, 1995; Girin & Boulétreau, 1995; Vavre, Girin & Boulétreau, 1999). The consequence of *Wolbachia* infection on host fitness thus remains unclear. Most data are only by-products of experiments dealing with the effects of *Wolbachia* on host reproduction and we lack careful studies on the physiological cost of infection.

Here, we have investigated the physiological cost that a parasitic wasp suffers when infected by a CI-inducing *Wolbachia*. Female *Leptopilina heterotoma* (Hymenoptera: Figitidae) deposit their eggs in the body of *Drosophila* larvae which are then used for parasitic development (Carton *et al.* 1986). In this wasp species, all individuals have been shown to be infected by 3 *Wolbachia* variants whose respective roles in CI remain unknown (Vavre *et al.* 1999). In contrast with previous investigations on the cost of *Wolbachia* infection, we were not content with effects on the classical components of wasp fitness such as fecundity and adult survival, but we also analysed behavioural traits, which surprisingly have not been so far considered despite their importance in reproductive success. We chose locomotor activity, a good indicator of the overall physiological state of individuals, and circadian rhythm, which determines the temporal organization of behaviours and thus constitutes an important component of fitness (Daan, 1984; Pittendrigh, 1993; Fleury *et al.* 2000). Here, we show that *Wolbachia* have deleterious effects on several fitness traits in *L. heterotoma* including locomotor activity in both sexes, thus suggesting heavy physiological cost of infection.

MATERIALS AND METHODS

Strains, rearing and experimental conditions

Effects of *Wolbachia* on host fitness were investigated by comparing a naturally infected *L. heterotoma* line and a derived uninfected subline obtained by curing individuals with 0.2% rifampicin antibiotic treatment over 3 consecutive generations (see Vavre *et al.*

2000). The aposymbiotic line was then cultured without antibiotics and no restoration of infection has been observed (20 generations). We used a highly inbred homozygous line (A7) originating from a strain caught in Antibes (south France), which was kept under regular sib-mating for 35 generations. This allows us to compare infected and uninfected individuals with exactly the same nuclear background, and prevents any interference of infection status with possible genetic divergence between infected and uninfected lines. All parasitoid lines were reared on a *Wolbachia*-free strain of *Drosophila melanogaster* fed standard diet (David, 1962). Experiments were performed 20 generations after antibiotic treatment stopped, and thus any direct deleterious effects of antibiotic exposure can be discarded. Experiments and rearing were carried out at 22 °C under LD 12:12 and 70% R.H.

Locomotor activity rhythms

Individual locomotor activity rhythms were monitored using a video-tracking and image analysis system which allows automatic continuous measurement of 120 insects over several days (Allemand *et al.* 1994). Individuals were isolated in experimental circular glass arenas without hosts but with honey as food. The locomotor activity of each individual was quantified every 6 min by binary data (1 if wasp has moved during a 2-sec video recording and 0 if not) and hourly activity was calculated as the percentage of active recordings among the 10 hourly ones. Infected and uninfected individuals (30 of both sexes) were measured 3 days under LD 12:12 (entrained rhythm), and then under constant darkness (DD) during 7 days (free-running experiment). Measurement in the absence of periodical environmental cues reveals the period of endogenous clock τ (see Saunders, 1982). Under both LD and DD conditions, the average daily pattern of activity was determined for each individual and 2 independent parameters were estimated: the rate of locomotor activity (RLA), calculated as the mean percentage of active recordings, and the profile of the rhythm, which gives the pattern of the hourly percentage of total daily activity. RLA measures the locomotor performance of wasps, whereas the profile of the rhythm illustrates the daily organization of activity which is basically bimodal in *L. heterotoma* (Fleury *et al.* 1995). Curves were plotted as a 3-point moving average to better determine time (phase) of activity peaks. Under LD 12:12, data are given as a function of Zeitgeber time (time of environmental cycle, Zt in hours) where light is turned on at Zt12 and off at Zt0. Under DD, activity is plotted as a function of Circadian time (Ct), each circadian unit being equivalent to $\tau/24$ h where τ is the measured period of free-running rhythm. This period was determined by the Chi-square periodogram method (Sokolove &

Table 1. Parameters of locomotor activity rhythms under LD 12:12 in infected and uninfected individuals

(Data were analysed using a two-way ANOVA and significant effects are given in bold. Rate of locomotor activity (RLA) was arcsine square root transformed prior to ANOVA analysis. Zt = hours in Zeitgeber time with light-on at Zt 12 and light-off at Zt 0. s.d., standard deviation.)

	Morning peak (Zt)	Evening peak (Zt)	RLA (%)
Mean \pm s.d. (N)			
Male I	12.1 \pm 0.7 (30)	22.1 \pm 0.7 (30)	8.8 \pm 6.3 (30)
Male U	12.3 \pm 0.8 (30)	22.1 \pm 0.6 (29)	13.6 \pm 8.0 (30)
Female I	12.6 \pm 0.6 (29)	22.0 \pm 0.7 (26)	9.6 \pm 6.8 (28)
Female U	12.4 \pm 0.7 (30)	21.9 \pm 0.8 (30)	13.2 \pm 8.6 (30)
F of ANOVA (p)			
Infection	0.01 (0.94)	0.6 (0.45)	9.5 (0.003)
Sex	5.9 (0.02)	1.5 (0.21)	0.02 (0.88)
Interaction	3.6 (0.06)	0.3 (0.60)	0.2 (0.64)
D.F.	(1,115)	(1,111)	(1,114)

Bushell, 1978) and most often differs from 24 h. RLA of infected and uninfected individuals were arcsine square root transformed prior to ANOVA analysis. Since curves of the profile of the rhythm become completely unclear when error bars are given for each hour, we thus chose to calculate the average confidence interval (aci) which is the mean of confidence intervals of all active points of each curve.

Development time and sex-ratio

Infected and uninfected parasitoids were cultured in vials (10 vials per line) containing 15 g of axenic medium (David, 1962). In each vial, 4 females were allowed to parasitize 150 *Drosophila* larvae during 24 h. The parasitization rate of *Drosophila* larvae is high and provides large numbers of adult parasitoids having developed without resource limitation (no competition). After development (22 °C, LD 12:12), newly emerged parasitoids were collected every day, sexed and counted. For each vial we calculated the mean development time of sexes (80–120 individuals per vial), and the sex-ratio (% males). A two-way ANOVA was performed on the means per vial. Sex-ratio analyses were carried out after arcsine square root transformation.

Fecundity

Fecundity was estimated by the number of eggs in ovaries. Since this species is proovigenic (no egg production during the adult stage), egg load gives a

good estimation of the reproductive potential of females. Newly emerged females were kept 5 days in vials containing honey as food to allow all eggs to mature. Each female was then etherized and dissected in a drop of physiological solution. One ovary was transferred for 5 min into neutral red solution and eggs were carefully scattered between slide and cover-glass. Coloured eggs were counted under the microscope with the help of a video system. Fecundity was estimated as twice the egg load in 1 ovary.

Adult survival without food

Because the life-span of fed wasps can reach several months, we measured adult survival under starvation. After development under controlled conditions (no competition, 22 °C, LD 12:12), 2-h-old individuals were placed in vials containing humidified cotton but neither food nor host. In each line and each sex, 10 vials containing 5 individuals were kept until all the wasps died. Mortality was checked every day and we calculated the mean individual life-span in each sex. A two-way ANOVA was performed on the means per vial.

RESULTS

Wolbachia infection modifies most fitness components studied in *L. heterotoma*. Tables 1 and 2 summarize the effects of infection on locomotor activity rhythms and Table 3 on other fitness traits.

Table 2. Parameters of locomotor activity rhythm measured under constant darkness (free-running experiment) for infected and uninfected individuals

(Data were analysed using one or two-way ANOVA (significant effects in bold). Rate of locomotor activity (RLA) was arcsine square root transformed prior to ANOVA analysis. Ct = time in Circadian time (circadian period of the endogenous clock τ divided by 24, see text.)

	Morning peak (Ct)	Evening peak (Ct)	Period (τ) (hours)	RLA (%)
Mean \pm s.d. (N)				
Male I	13.4 \pm 1.1 (28)	—	23.21 \pm 2.42 (28)	7.8 \pm 3.6 (28)
Male U	13.6 \pm 1.6 (22)	—	23.28 \pm 2.50 (22)	11.7 \pm 5.4 (28)
Female I	15.4 \pm 1.6 (26)	21.36 \pm 1.54 (28)	22.93 \pm 3.23 (29)	25.0 \pm 9.2 (29)
Female U	14.8 \pm 1.1 (29)	21.59 \pm 1.15 (29)	22.96 \pm 2.73 (30)	34.2 \pm 9.3 (30)
F of ANOVA (p)				
Infection	0.4 (0.51)	0.4 (0.52)	0.8 (0.37)	6.3 (0.001)
Sex	35.9 (< 0.001)	—	33.2 (< 0.001)	22.8 (< 0.001)
Interaction	2.9 (0.09)	—	0.13 (0.71)	3.6 (0.06)
D.F.	(1,101)	(1,55)	(1,105)	(1,113)

Table 3. Effect of *Wolbachia* infection on fitness components of *Leptopilina heterotoma*

(Means of each parameter (\pm standard error) are given with results of one or two-way ANOVA (significant effects in bold). Sex-ratio was arcsine square root transformed prior to ANOVA analysis.)

	Fecundity (Nb eggs)	Sex-ratio (% males)	Adult survival (days)	Development time (days)
Mean \pm s.d. (N)				
Male I	—	—	7.2 \pm 0.36 (10)*	25.0 \pm 0.15 (10)†
Male U	—	—	7.7 \pm 0.46 (10)*	24.9 \pm 0.16 (10)†
Female I	304.6 \pm 31.4 (30)	0.56 \pm 0.09 (10)†	9.9 \pm 0.49 (10)*	27.3 \pm 0.21 (10)†
Female U	331.6 \pm 43.2 (30)	0.56 \pm 0.12 (10)†	10.3 \pm 0.39 (10)*	27.4 \pm 0.32 (10)†
F of ANOVA (p)				
Infection	7.7 (0.008)	0.01 (0.99)	12.4 (0.001)	0.01 (0.94)
Sex	—	—	381 (< 0.0001)	114.2 (< 0.0001)
Interaction	—	—	0.4 (0.56)	0.4 (0.54)
D.F.	(1,58)	(1,18)	(1,36)	(1,36)

* 10 vials of 5 individuals.

† 10 vials of at least 70 individuals.

The most significant effect of *Wolbachia* was on the locomotor performance of wasps (Fig. 1). Under LD 12:12, infection results in reduced RLA in both males (-35%) and females (-27%) (Table 1). Both sexes are similarly affected (no significant sex-infection interaction). In contrast, *Wolbachia* infection does not change the profile of the rhythm which is identical in infected and uninfected individuals. Figure 1 gives the average daily pattern of

activity in both sexes, and shows that the temporal organization of activity is bimodal with 1 peak at light-on and 1 before light-off. The phase of these peaks is the same in cured and control lines and the only significant effect was among sexes for the first peak, slightly earlier in males (Table 1).

Results under LD are fully confirmed by the free-running experiment carried out in constant darkness which reveals the endogenous basis of locomotor

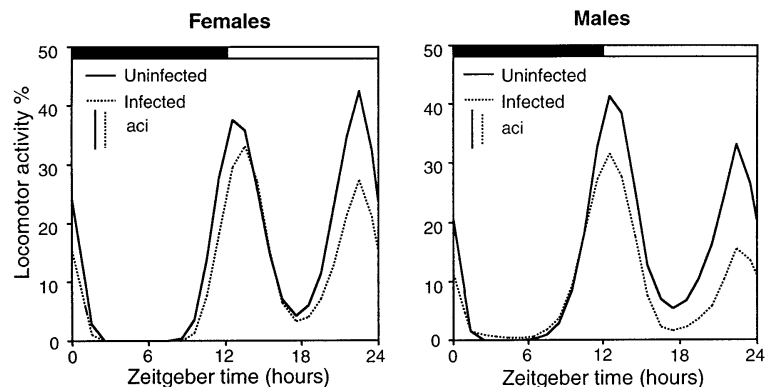


Fig. 1. Mean curves of locomotor activity rhythms of infected and uninfected *Leptopilina heterotoma*. Males and females were measured for 3 days under LD 12:12 (Zeitgeber time) with light-on at Zt12 and light-off at Zt0. Black and white rectangles on the top of the figure represent the night and the day respectively. aci = average confidence interval (see text).

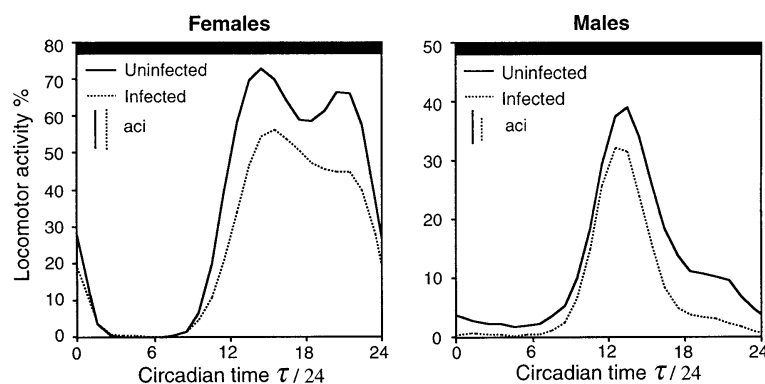


Fig. 2. Mean curves of free-running rhythms of infected and uninfected *Leptopilina heterotoma*. Males and females were measured for 7 days under constant darkness. Activity is plotted against Circadian time (circadian period of the endogenous clock τ divided by 24, see text). aci = average confidence interval.

activity rhythms (Fig. 2 and Table 2). The stability of rhythm under constant darkness demonstrates that the daily pattern of activity is controlled by an endogenous programme or biological clock, thus confirming previous results (Fleury *et al.* 1995). The main parameters of these endogenous oscillations are not affected by *Wolbachia* infection, particularly the period of the endogenous rhythm which differs significantly from 24 h (circadian rhythm) (Table 2). Similar results were observed on the phases of the rhythm which only vary between sexes. Phase angle between the first peak of activity and the light-off signal is shorter in males, which could explain their earlier activity in LD. It is also noteworthy that the daily pattern of male activity shifts from bimodal under LD to unimodal under constant darkness, thus suggesting that male rhythm has basically only 1 peak of activity and that the second bout of activity under LD has exogenous determinism. Under constant darkness, the only significant effect of *Wolbachia* infection is on RLA, which is 30% reduced in infected individuals of both sexes (Fig. 2). The persistence under constant darkness of lower locomotor performance in infected individuals indi-

cates that *Wolbachia* do not modify the response to the light signal, but rather affect the whole host physiology.

Among the 4 conventional components of insect fitness here studied, development time and sex-ratio do not differ between infected and uninfected parasitoids, whereas both fecundity and adult survival are affected (Table 3). At 22 °C, egg to adult development time is significantly shorter in males (2–3 days) as already known in this species (Carton *et al.* 1986), but *Wolbachia* have no effect on the trait. Offspring sex-ratio is the same for infected and uninfected females (56% males). In contrast, *Wolbachia* significantly reduce adult survival in both sexes. In the absence of food, the life-span of uninfected wasps is, on average, 10 days for females, 7.7 for males. In infected wasps, survivorship is reduced by half a day in both sexes (no infection \times sex interaction). This difference in adult survival is weak (6%) but highly significant, thus demonstrating that *Wolbachia* induce a physiological cost to the host. This is confirmed by the change in fecundity of females. Infected females produce on average 27 eggs fewer than aposymbiotic ones (–8%).

Altogether, the results clearly show that *Wolbachia* infection reduces the fitness of female parasitoids, mainly their fecundity and survivorship.

DISCUSSION

Among the 6 traits studied in *Leptopilina heterotoma*, 3 proved affected by infection (adult survival, fecundity and locomotor activity) while 3 remained unchanged (development time, sex ratio and circadian organization of activity). This contrasts with previous studies which either failed to demonstrate any fitness effect of *Wolbachia* infection (Hoffmann *et al.* 1994; Turelli & Hoffmann, 1995; Poinot & Merçot, 1997), or evidenced either positive or negative effects depending on the fitness traits studied (Hoffmann *et al.* 1990; Wade & Chang, 1995). If we except the special case of the popcorn variant which kills its adult *Drosophila* host (Min & Benzer, 1977), *Wolbachia* inhabiting *L. heterotoma* appear as one of the most pathogenic so far described in insects. Decrease in fecundity (8%) is consistent with that described in *Drosophila simulans* (Hoffmann *et al.* 1990) and clearly results in fitness reduction. The most detrimental effect occurs on the rate of locomotor activity, which shows reduction in both sexes without changes in the circadian pattern. This reduction suggests that bacteria interact with energy metabolism of infected adults, either through reduction in their gross energy budget, or through release of toxic compounds. Since locomotion is directly linked to most behavioural fitness components (Partridge, Ewing & Chandler, 1987; Bigler *et al.* 1988; Bell, 1991; Ruiz-Dubreuil, Burnet & Connolly, 1994), this reduced locomotor activity of infected parasitoids probably has negative consequences on their fitness such as resource finding efficiency or ability to locate sexual partners. The behavioural impact of *Wolbachia* infection remains poorly documented despite its possible consequence on the mating success of infected males and the resulting frequency of incompatible crosses. This could also have consequences for the dynamics of *Wolbachia* invasion which is highly sensitive to infection cost (Turelli, 1994; Vavre *et al.* 2000).

We can wonder about this virulence, since vertically transmitted symbionts are expected to evolve toward mutualism (Anderson & May, 1982; Smith & Dunn, 1991; Ebert & Herre, 1996). However, due to their effects on host reproduction, *Wolbachia* can be considered as departing from this common rule. Cytoplasmic incompatibility puts infected females at an advantage over uninfected ones, and thus favours bacterial spread into the host population despite their physiological cost. Usually, this deleterious effect is absent or light (Hoffmann & Turelli, 1997), and its severity in *L. heterotoma* can be explained in several ways. Heavy cost can be ascribed to high bacterial density which could result from the novelty

of the *Wolbachia*-*L. heterotoma* association with a lack of coadaptation between the host's and the bacterial genomes. This could explain high transmission efficiency and total cytoplasmic incompatibility in this species (Vavre *et al.* 2000) counterbalancing the cost of infection. However, since selection acting on both host and bacteria should lead to reduced physiological cost (Turelli, 1994), it is possible that the present association is in a transient evolutionary stage. An alternative would be to consider that evolutionary stable situations can be reached with high infection levels and complete transmission efficiency through high bacterial densities, despite the corresponding physiological cost. The last explanation results from theoretical considerations predicting that while parasites should evolve to avirulence when alone, conflicts of interest occurring among different parasites (genotypes or species) that share the same host individual should favour the most virulent strategy (Levin & Pimentel, 1981; Van Baalen & Sabelis, 1995). Since in *L. heterotoma* all individuals prove infected by 3 different *Wolbachia* variants (Vavre *et al.* 1999), within-host competition among them could favour high bacterial densities and high physiological cost.

Actual field consequences of the deleterious effect of *Wolbachia* infection in *L. heterotoma* populations remain to be evaluated, but they could modify interactions with other species such as *Drosophila* hosts or other competing parasitoids. Depending on the local structure of host-parasitoid communities, *L. heterotoma* populations should evolve different relationships with *Wolbachia*, thus leading to local differentiation of wasp-bacteria relationships as suggested by preliminary results (unpublished). Finally, these results extend the phenotypic consequence of *Wolbachia* infection in insects, which proves particularly pathogenic in *L. heterotoma* but could be positive or neutral in other species (Hoffmann, Clancy & Duncan, 1996; Vavre, Girin & Boulétreau, 1999; Hoerauf *et al.* 1999). However, in most cases, fitness modification remains within a narrow range of variation around neutrality and *Wolbachia* thus appears as a useful model to study evolution of symbiosis.

We thank R. Allemand for useful discussion on results of these experiments and S. Martinez for rearing of *Drosophila* parasitoids. Financial support came from the European Commission (grant AIR CT 94-1433) and the Centre National de la Recherche Scientifique (programme PNDBE).

REFERENCES

- ALLEMAND, R., POMPANON, F., FLEURY, F., FOUILLET, P. & BOULÉTREAU, M. (1994). Behavioural circadian rhythms measured in real-time by automatic image analysis: application in parasitoid insects. *Physiological Entomology* **19**, 1–8.

- ANDERSON, R. M. & MAY, R. M. (1982). Coevolution of hosts and parasites. *Parasitology* **85**, 411–426.
- BANDI, C., ANDERSON, T. J. C., GENCHI, C. & BLAXTER, M. L. (1998). Phylogeny of *Wolbachia* in filarial nematodes. *Proceedings of the Royal Society of London, B* **265**, 2407–2413.
- BELL, W. J. (1991). *Searching Behavior*. Chapman and Hall, London.
- BIGLER, F., BIERI, M., FRITSCHY, A. & SEIDEL, K. (1988). Variation in locomotion between laboratory strains of *Trichogramma maidis* and its impact on parasitism of eggs of *Ostrinia nubilalis* in the field. *Entomologia Experimentalis et Applicata* **49**, 283–290.
- BREEUWER, J. A. J. & WERREN, J. H. (1990). Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature, London* **356**, 558–560.
- BOURTZIS, K., NIRGIANAKI, A., MARKAKIS, G. & SAVAKIS, C. (1996). *Wolbachia* infection and cytoplasmic incompatibility in *Drosophila* species. *Genetics* **144**, 1063–1073.
- CARTON, Y., BOULÉTREAU, M., VAN ALPHEN, J. J. M. & VAN LENTEREN, J. C. (1986). The *Drosophila* parasitic wasps. In *The Genetics and Biology of Drosophila* (ed. Ashburner, M., Thompson, H. L. & Carson, J. N.), pp. 347–394. Academic Press, London.
- CLANCY, D. J. & HOFFMANN, A. A. (1997). Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *D. serrata*, a novel host. *American Naturalist* **149**, 975–988.
- COOK, J. M. & BUTCHER, R. D. J. (1999). The transmission and effects of *Wolbachia* bacteria in parasitoids. *Research Population Ecology* **41**, 15–28.
- DAAN, S. (1984). Adaptive daily strategy in behavior. In *Handbook of Behavioral Neurobiology, Vol. 4, Biological Rhythms* (ed. Aschoff, J.), pp. 275–298. Plenum Press, New York.
- DAVID, J. R. (1962). A new medium for rearing *Drosophila* in axenic condition. *Drosophila Information Service* **36**, 128.
- EBERT, D. & HERRE, E. (1996). The evolution of parasitic diseases. *Parasitology Today* **12**, 96–101.
- FLEURY, F., ALLEMAND, R., FOUILLET, P. & BOULÉTREAU, M. (1995). Genetic variation in the locomotor activity rhythm among populations of *Leptopilina heterotoma* (Hymenoptera: Cynipidae), a larval parasitoid of *Drosophila* species. *Behavior Genetics* **25**, 81–89.
- FLEURY, F., ALLEMAND, R., VAVRE, F., FOUILLET, P. & BOULÉTREAU, M. (2000). Adaptive significance of a circadian clock: temporal segregation of activities reduces intrinsic competitive inferiority in *Drosophila* parasitoids. *Proceedings of the Royal Society of London, B* **267**, 1005–1010.
- GIORDANO, R., O'NEILL, S. L. & ROBERTSON, H. M. (1995). *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. simulans*. *Genetics* **140**, 1307–1317.
- GIRIN, C. & BOULÉTREAU, M. (1995). Microorganism associated variation in host infestation efficiency in a parasitoid wasp, *Trichogramma bourarachae* (Hymenoptera Trichogrammatidae). *Experientia* **51**, 398–401.
- HOERAUF, A., NISSEN-PÄHLE, K., SCHMETZ, C., HENKLE-DÜHRSEN, K., BLAXTER, M. L., BÜTTNER, D. W., GALLIN, M. Y., AL-QAOD, K. M., LUCIUS, R. & FLEISCHER, B. (1999). Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *Journal of Clinical Investigation* **103**, 11–17.
- HOFFMANN, A. A., CLANCY, D. J. & DUNCAN, J. (1996). Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. *Heredity* **76**, 1–8.
- HOFFMANN, A. A., CLANCY, D. J. & MERTON, E. (1994). Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. *Genetics* **136**, 993–999.
- HOFFMANN, A. A., HERCUS, M. & DAGHER, H. (1998). Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* **148**, 221–231.
- HOFFMANN, A. A. & TURELLI, M. (1997). Cytoplasmic incompatibility in insects. In *Influential Passengers* (ed. O'Neill, S. L., Hoffmann, A. A. & Werren, J. H.), pp. 42–80. Oxford University Press, Oxford.
- HOFFMANN, A. A., TURELLI, M. & HARSHMAN, L. G. (1990). Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* **126**, 933–948.
- HURST, G. D. D., JIGGINS, F. M., SCHULENBERG, J. H. G., BERTRAND, D., WEST, S. A., GORIACHEVA, I. I., ZAKHAROV, I. A., WERREN, J. H., STOUTHAMER, R. & MAJERUS, M. E. N. (1999). Male-killing *Wolbachia* in two species of insect. *Proceedings of the Royal Society of London, B* **266**, 735–740.
- LEVIN, S. & PIMENTEL, D. (1981). Selection of intermediate rates of increase in parasite-host systems. *The American Naturalist* **117**, 308–315.
- MIN, K. T. & BENZER, S. (1997). *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proceedings of the National Academy of Sciences, USA* **94**, 10792–10796.
- O'NEILL, S. L. (1995). *Wolbachia pipientis*: symbiont or parasite? *Parasitology Today* **11**, 168.
- O'NEILL, S. L., HOFFMANN, A. A. & WERREN, J. H. (1997). *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford University Press, Oxford.
- PARTRIDGE, L., EWING, A. & CHANDLER, A. (1987). Male size and mating success in *Drosophila melanogaster*: the role of male and female behavior. *Animal Behavior* **35**, 555–562.
- PITTENDRIGH, C. S. (1993). Temporal organization: reflections of a Darwinian clock-watcher. *Annual Review of Physiology* **55**, 17–54.
- POINSOT, D. & MERÇOT, H. (1997). *Wolbachia* infection in *Drosophila simulans*: Does the female host bear a physiological cost? *Evolution* **51**, 180–186.
- RIGAUD, T. (1997). Inherited microorganisms and sex determination of arthropod hosts. In *Influential Passengers* (ed. O'Neill, S. L., Hoffmann, A. A. & Werren, J. H.), pp. 81–101. Oxford University Press, Oxford.
- RUIZ-DUBREUIL, G., BURNET, B. & CONNOLLY, K. (1994). Behavioural correlates of selection for oviposition by *Drosophila melanogaster* females in a patchy environment. *Heredity* **73**, 103–110.
- SAUNDERS, D. S. (1982). *Insect Clocks*. 2nd Edn. Pergamon Press, Oxford.

- SMITH, J. F. & DUNN, A. M. (1991). Transovarial transmission. *Parasitology Today* **7**, 146–148.
- SOKOLOVE, P. G. & BUSHELL, W. N. (1978). The Chi square periodogram: its utility for analysis of circadian rhythms. *Journal of Theoretical Biology* **72**, 131–160.
- STOUTHAMER, R. (1997). *Wolbachia*-induced parthenogenesis. In *Influential Passengers* (ed. O'Neill, S. L., Hoffmann, A. A. & Werren, J. H.), pp. 102–124. Oxford University Press, Oxford.
- STOUTHAMER, R., LUCK, R. F. & HAMILTON, W. D. (1990). Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera: Trichogrammatidae) to revert to sex. *Proceedings of the National Academy of Sciences, USA* **87**, 2424–2427.
- TURELLI, M. (1994). Evolution of incompatibility-inducing microbes and their hosts. *Evolution* **48**, 1500–1513.
- TURELLI, M. & HOFFMANN, A. A. (1995). Cytoplasmic incompatibility in *Drosophila simulans*: dynamic and parameter estimates from natural populations. *Genetics* **140**, 1319–1338.
- VAN BAALEN, M. & SABELIS, M. W. (1995). The dynamics of multiple infection and the evolution of virulence. *The American Naturalist* **146**, 881–910.
- VAVRE, F., FLEURY, F., LEPETIT, D., FOUILLET, P. & BOULÉTREAU, M. (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host–parasitoid associations. *Molecular Biology and Evolution* **16**, 1711–1723.
- VAVRE, F., FLEURY, F., VARALDI, J., FOUILLET, P. & BOULÉTREAU, M. (2000). Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects, epidemiologic and evolutionary consequences. *Evolution* **54**, 191–200.
- VAVRE, F., GIRIN, C. & BOULÉTREAU, M. (1999). Phylogenetic status of a fecundity-enhancing *Wolbachia* that does not induce thelytoky in *Trichogramma*. *Insect Molecular Biology* **8**, 67–72.
- WADE, M. J. & CHANG, N. W. (1995). Increased male fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. *Nature, London* **373**, 72–74.
- WERREN, J. H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology* **42**, 587–609.
- WERREN, J. H., ZHANG, W. & GUO, L. R. (1995). Evolution and phylogeny of *Wolbachia*: reproductive parasite of arthropods. *Proceedings of the Royal Society of London, B* **251**, 55–71.