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Microorganism-associated variation in host infestation efficiency in a parasitoid wasp, *Trichogramma bourarachae* (Hymenoptera: Trichogrammatidae)

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Abstract. In *Trichogramma bourarachae* (Hymenoptera: Trichogrammatidae), the infestation efficiency presents important variation among strains: 60 eggs/5 days/female in the High (H) strain and 25 eggs/5 days/female in the Low (L) strain. Crosses show that between-strains variation is inherited by the matrocline route. Antibiotic and heat treatments reduce infestation efficiency in the H strain and have no effect on the L strain. The hypothesis of cytoplasmic-symbiotic microorganisms accounting for the higher infestation efficiency in the H strain is supported by microscopic observations. The origin and the significance of this between-strains variation are discussed.

Key words. *Trichogramma bourarachae*; parasitoids; insects; reproduction; heat treatment; antibiotic treatment; maternal inheritance; symbionts.

In parasitoid insects several cases of association with microbes have been reported recently. In Ichneumonidae, polyDNA virus-like-particles are responsible for immunodepression in the host, which protects the parasite from the cellular immune reaction[1,2]. In Chalcididae, extra chromosomal factors regulate parthenogenesis in *Nasonia*[3]. In several *Trichogramma* species[4,5], thelytoky* is due to symbiotic microorganisms. By curing the asexual strains, either by heat or by antibiotics, arrhenotoky* is restored. These microbes are *Rickettsia* of the genus *Wolbachia*[6,7].

In 1991, Mimouni[8] reported the maternal inheritance of variation in fecundity in two arrhenotokous *Trichogramma* species from Morocco, *T. bourarachae* and *T. voegelei*, and suggested the possible role of microbes. The experiments reported here confirm the maternal inheritance of variation in fecundity in *T. bourarachae*, and demonstrate that the higher fecundity in the more fecund strain is due to the presence of a favourable symbiotic microorganism.

Material and methods

Strains. We used two haplo-diploid Moroccan strains of *T. bourarachae*: the High strain (H), founded in 1988 from parasitized *Vanessa cardui* eggs found in Tadla (near Marrakech), and the Low strain (L) caught in 1984 in El Oualidia (Atlantic coast) on *Heliothis armigera* eggs. These strains have been defined by their different host infestation efficiencies: 61.3 ± 1.4/female/5 days ($n = 7$), vs 25.8 ± 1.8 ($n = 20$). They show similar egg laying kinetics and have equal life times. Since their capture both strains have been kept in the lab under mass-rearing conditions (at least 500 indiv./generation), using *Ephestia kuehniella* eggs as hosts.

Measures of infestation efficiency. To measure the host infestation efficiency, isolated young females (less than 24 h old) were exposed for 5 days to a clutch of about 500 UV-killed *Ephestia* eggs, glued on to a 1 × 1 cm cardboard sheet. Parasitized host eggs turn blackish after 4 or 5 days and can be counted easily. It is known that the number of hosts that a female infests within the first five days of its life is highly representative of its total fecundity[9]. All measurements were performed at 22 °C, 50% RH under LD 12:12 photoperiod.

Experiments. The following treatments (heat and antibiotic) were applied to females (mothers). Untreated female first day offspring were then individually tested for their infestation efficiency.

Heat treatment applied to mothers. In each strain (H and L), 20 pairs were chosen at random just after emergence. Ten of them were placed at 30 °C for one day, then transferred to 22 °C. Ten others were held at 22 °C as controls. All females were fed honey throughout the experiment and individually provided with hosts from the second day.

Antibiotic treatments applied to mothers. Tetracycline (T: a first generation antibiotic; Sigma®) or doxycyline (a recent antiobiotic; Vibraveineuse, Pfizer®) was added to honey to a concentration of 0.25%. Emergent females (mothers) were randomly crossed with males of the same strain, then provided with host eggs. They were fed either pure honey (10 control females/strain), or antibiotic-supplemented honey (10 females/strain) for one day, then pure honey.

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* thelytoky: obligatory parthenogenesis in which a female gives rise only to female offspring.
arrhenotoky: parthenogenesis in which unfertilized eggs produce haploid males and fertilized eggs diploid females.

Table 1. Effect of antibiotics on infestation efficiency.

		Controls	Tetracycline	Doxycycline	Analysis of variance
Mothers F0	H	57.6 ± 1.67	56.5 ± 0.91	58.3 ± 1.53	NS
	L	22 ± 1.34	23.4 ± 0.75	23.1 ± 0.81	NS
Daughters F1	H	58.7 ± 1.01 a	46.5 ± 2.15 b	33.3 ± 1.7 c	p = 0.0001
	L	23.7 ± 1.01	22.6 ± 2.53	20.2 ± 0.90	NS

Antibiotic treatments are applied to females (mothers F0) of the H and the L strains with 2 substances (tetracycline and doxycycline) at a dose of 0.25%. Their daughters (F1) are all untreated. For mothers and daughters, the mean (± standard error) of each group (control, tetracycline, doxycycline) is calculated on 10 females, the 3 means are compared with an analysis of variance. The different lower-case letters show that the means are significantly different.

Table 2. Effect of heat on infestation efficiency.

		Controls (22 °C)	Heat treated (30 °C)	Analysis of variance
Mothers F0	H	54.1 ± 1.87	45.7 ± 1.76	p = 0.0043
	L	26.3 ± 2.11	20.1 ± 1.26	p = 0.021
Daughters F1	H	56.7 ± 1.13	44.8 ± 2.02	p = 0.0001
	L	25.5 ± 0.43	23.6 ± 0.81	NS

Heat treatments (30 °C) are applied to females (mothers F0) of the H and the L strains. Their daughters (F1) are *all* untreated. For mothers and daughters the mean (± standard error) is calculated on 10 females, means are compared using an analysis of variance.

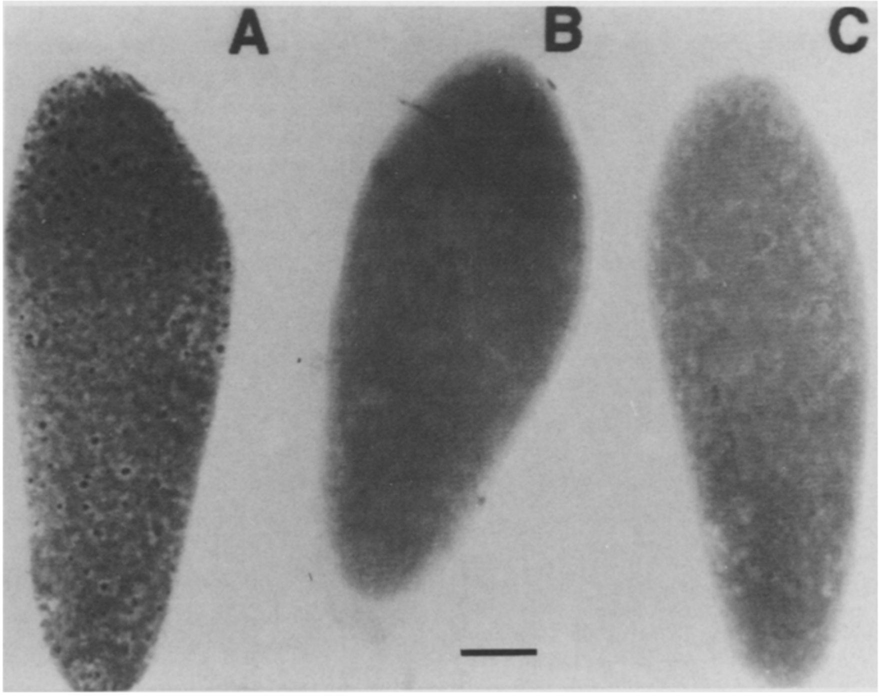

Figure 1. Microscopic observations showing the eggs of *Trichogramma bourarachae* (lacmoid staining[10]). Eggs of the H strain with microorganisms (A), eggs free of microorganisms of the L strain (B) and of the HT strain (H strain treated with tetracycline), (C); (bar = 25 μm).

Measurements on daughters. In all groups, one daughter was randomly chosen among the first day offspring of each treated or untreated female. These daughters were individually tested for their host infestation efficiency according to the above procedure.

Crosses and back-crosses. These were only performed in the doxycycline experiment. In each of the H and L strains and among the daughters of the antibiotic-treated H females (HT), 15 emergent females were individually crossed with 15 males originating either from

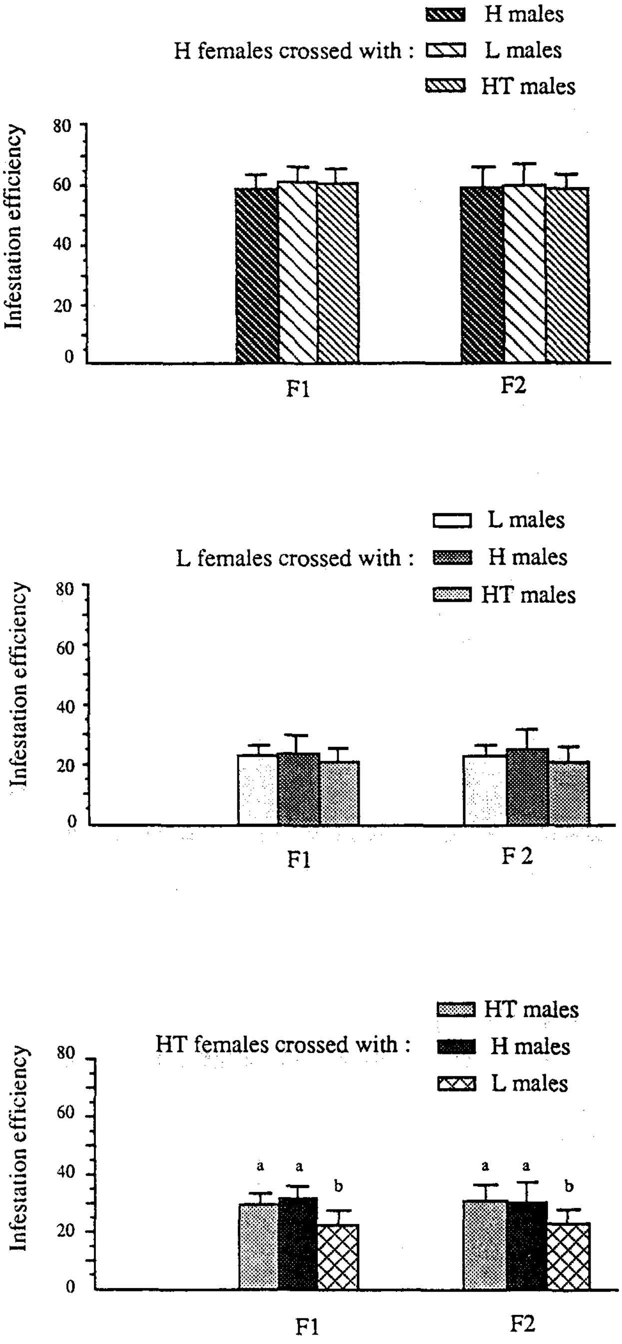

Figure 2. Inheritance of infestation efficiency. Crosses are made between original strains (H and L) and H strain treated with doxycycline (HT). The diagrams present the infestation efficiency per female at 5 days for the first (F1) and for the second (F2) generation. Every column is a mean (±SD) of 15 females' infestation efficiency. For HT females crossed with HT, H and L males, the means are compared separately for the F1 and F2 with analysis of variance. The different lower-case letters show that means are significantly different.

strains H, L, or HT, fed honey and individually provided with hosts (9 combinations, 15 replicates each, 135 progenies analysed). In each of the 15 F1 progenies obtained within each of the 9 crossing treatments, one daughter of a one-day-old mother was chosen and individually back-crossed with one male of the same strain as its father. In the next generation, F2 females were individually back-crossed with males of the same strain as their grandfathers, according to the same procedure as in the previous generation (15 replications in each of 9 crossing treatments, 135 progenies).

Staining. Eggs from each *Trichogramma* strain (H, L, and the tetracycline-treated H strains [HT]) were obtained by dissection of parasitized hosts and stained with a lacmoid solution[10].

Results

Antibiotics and heat effects. The fecundity of the treated females was not affected by antibiotics (table 1), but it was reduced by heat treatment (table 2). In the L strain, the fecundity of daughters was not affected by the antibiotics or heat treatment their mother received. By contrast, the H strain proved sensitive. The fecundity of daughters was significantly reduced by antibiotic treatment of their mother, though it was not reduced to the level of the L strain. Doxycycline was far more efficient than tetracycline, probably due to its better cellular penetration ability (table 1). Heating mothers for 24 hours reduced the fecundity of their daughters significantly (table 2).

Thus the two strains differed considerably in their sensitivity to treatments that are well known for their curative effects on symbionts. The three treatments tested here – heat, doxycycline and tetracycline – were not equally efficient. We have indications that more prolonged treatments with tetracycline or heat have a stronger effect, but also interfere with ageing of the mothers.

Clearly, the higher fecundity of the H strain can be ascribed to symbiotic agents, and this experimental conclusion is fully confirmed by microscopic observations (fig. 1). The eggs of the H strain contained microorganisms, concentrated just below the vitelline membrane, variable in shape and size, that were absent from the eggs of the L strain and the eggs of the H strain after tetracycline treatment.

Inheritance of infestation efficiency (fig. 2). In crosses involving females originating from the H or L strain, the infestation efficiency was stable over generations whatever males were used. The absence of an increase in fecundity in the H × L crosses shows that the differences between parental strains did not originate from their different amounts of inbreeding depression due to their different histories. Moreover, the fecundity of F1 and F2 females was entirely dependent on the origin of the initial females, and the partial substitution of the maternal genome by the paternal one did not affect the infestation efficiency.

Similarly, crosses between HT females and H males and further back-crosses with H males did not restore the oviposition rate of the offspring, thus confirming the existence of a strong maternal influence. However, the L paternal genome was able to slightly reduce the infestation efficiency of the doxycycline treated HT females, thus demonstrating a host nuclear genomic influence.

Conclusions

Clearly, variation in host infestation efficiency among strains of *T. bourarachae* has a cytoplasmic basis, and furthermore, the greater efficiency of the H strain is due to the presence of symbionts.

This strong cytoplasmic influence conceals a slight host nuclear genomic effect, which expressed itself neither in crosses involving H females, nor in crosses involving the naturally asymbiotic L ones, but only in crosses involving the artificially cured HT females. An explanation could be that the antibiotic treatment did not eliminate all symbionts (see the higher fecundity of HT vs L females, cf. table 1), and that the L genome is less suited to colonization by the symbionts. Therefore the phenotypic effect of the H/L heterozygosity could be significant when associated with the HT cytoplasm where symbionts are scarce, but undetectable both in the symbiont-free L cytoplasm, and in the H one which could remain rich in symbionts for a few generations. Current experiments are comparing the long-term stability of the association of H cytoplasm with different combinations of H and L nuclear genomes.

An influence of symbionts on fecundity is not uncommon in insects, but up to now it had never been reported in a parasitoid species. A number of questions arise concerning this new host-symbiont system (localization, transmission, etc), and we will only discuss a few of them.

What are these microbes? We have only weak and indirect arguments for their Rickettsian nature: their sensitivity to tetracycline[4,11] and their stainability by lacmoid solution[11]. Further analyses will explore their relationships with the *Wolbachia* which are responsible for thelytoky in other *Trichogramma* species[7].

How do they act on host infestation efficiency? The number of hosts that *Trichogramma* females can infest when hosts are unlimited depends on their egg production capacity, and we can presume that the symbionts increase egg production in the H strain. In *Trichogramma* species, most of the vitellogenesis is completed at the time of emergence, and takes place during the nymphal stage at the expense of larval reserves (proovogenic oogenesis)[12,13]. Thus symbionts could act predominantly during the larval, parasitic stage, and they would be directly involved in the host-parasite physiological and nutritional relationships[14,15].

How can we explain the existence of symbiotic and aposymbiotic strains before any deliberate curing has taken place? The two strains tested here differed in their histories. The L strain was caught 4 years before the H one, and it could have lost its symbionts over generations of laboratory rearing. However, the H strain proved to be stable over time (1988–1993) and did not lose its efficiency. Thus the aposymbiotic status of the L-strain should probably be attributed not to its long artificial rearing period, but rather to its different geographic origin (atlantic vs continental), and to its different genetic makeup. The two populations could have different reproductive strategies adapted each to a particular habitat, with both genetic and non-genetic determinisms, corresponding to differences in the balance of their fitness components. The existence of a population polymorphic for the presence of symbionts, currently under study, suggests the possibility that selection can act on this trait.

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