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RESPONSE TO SELECTION FOR A HIGH OR A LOW SEX RATIO IN *WOLBACHIA*-INFECTED LINES OF *TRICHOGRAMMA CORDUBENSIS* SUBJECTED TO HIGH TEMPERATURE

B. Pintureau, P. Bolland

Biologie Fonctionnelle, Insectes et Interactions — UMR INRA/INSA de Lyon, INSA Bâtiment L. Pasteur, 69621 Villeurbanne cedex, France
E-mail: pinture@jouy.inra.fr

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Response to Selection for a High or a Low Sex Ratio in *Wolbachia*-infected Lines of *Trichogramma cordubensis* Subjected to High Temperature. Pintureau B., Bolland P. — *Trichogramma cordubensis* is an egg parasitoid completely infected by endosymbiotic bacteria belonging to the *Wolbachia* genus and inducing the thelytokous mode of reproduction. Previous results showed that the thelytoky persistence at high temperature is genetically variable. Experimental selection was performed to confirm these results and to determine the possibilities of adaptation to the environment assuming cyclic bisexual reproduction. No selection was efficient and the genetic variability of the sex ratio at high temperature is probably lower than previously assumed. This reduces the adaptive possibilities in the studied *Trichogramma* species.

Key words: bacterium, egg parasitoid, Hymenoptera, parthenogenesis, symbiosis, thelytoky.

Возможности селекции линий с высоким и низким половым индексом у вида *Trichogramma cordubensis*, инфицированного бактерией *Wolbachia*, при воздействии высокой температуры. Пинтюро Б., Болланд П. — Установлено, что *Trichogramma cordubensis* — яйцевой паразит, характеризующийся наличием эндосимбионтных бактерий *Wolbachia* и телитокическим способом размножения. Отмечено, что устойчивость к высокой температуре у телитокических видов генетически варьирует. Проведена экспериментальная селекция линий с высоким и низким половым индексом для определения возможных адаптаций к условиям окружающей среды, вызывающим двуполый способ размножения. Установлено, что селекция выбранных линий была не эффективной. Генетически обусловленная изменчивость полового индекса при высокой температуре оказалась ниже, чем предполагалось. Отмечено, что это уменьшает адаптивные возможности *T. cordubensis*.

Ключевые слова: бактерии, яйцевой паразит, Hymenoptera, партеногенез, симбиоз, телитокия.

Introduction

The genus *Trichogramma* (Hym.: Trichogrammatidae) includes numerous species of egg parasitoids worldwide distributed and used in biological control against agricultural lepidopterous pests. Most of the species show a bisexual mode of reproduction and some species/populations have a parthenogenetic mode of reproduction (thelytoky) often induced by symbiotic bacteria belonging to the genus *Wolbachia*, and more precisely to the B supergroup of this genus (Rousset et al., 1992; Stouthamer et al., 1993; Zhou et al., 1998).

Trichogramma species are generally not completely *Wolbachia*-infected. However, the infection is complete in some totally thelytokous species as *T. cordubensis* Vargas & Cabello (Pintureau, 1994). In *T. cordubensis*, thelytoky shows a greater or lesser resistance to high temperature, and a part of this variability has a genetic origin (Pintureau et al., 1999). Bisexual reproduction can thus reappear more or less easily and the process allowing genetic recombinations could be selected to facilitate the adaptation of the population to the environment. The aim of the present work was to confirm or to refuse the possibility of such a process by means of experimental selection.

Material and methods

Experiments were carried out in the strain 1148 of *Trichogramma cordubensis* established from parasitoids collected in 1995 in S. Jorge Island, Azores. In the strain 802 from S. Miguel, Azores, the within- and between-line variances of the sex ratio (proportion of males) at 30°C, compared by an ANOVA, were significantly different (p=0.0001) (Pintureau et al., 1999). A genetic variability was thus present and assumed to be similar in the strain 1148.

Mass selection was applied to the character sex ratio or proportion of males. About 500 prenymphs of the thelytokous strain 1148, parasitizing *Ephestia kuehniella* (Lep.: Pyralidae) eggs, were placed at 29°C (F1 generation). The two subsequent generations (F2 and F3), also reared at 29°C, were established with a similar number of individuals. After emergence and mating, 100 females of the F3 were isolated in glass tubes where they were offered *E. kuehniella* eggs as hosts and a drop of diluted honey as food. The F4 progenies reared at 23°C were selected or not to establish three lines. The low line, i. e. the line established from the 10 progenies with the lower proportion of males, is expected infected by *Wolbachia* resistant to high temperature. The high line, i. e. the line established from the 10 progenies with the higher proportion of males, is expected infected by *Wolbachia* sensitive to high temperature. The control line was established from 10 progenies taken at random among those not selected for the preceding lines. To establish the high line, progenies with a sex ratio of 1 (only males) were rejected because they correspond to unmated aposymbiotic females. Progenies including less than 10 individuals were never taken into consideration.

The applied high temperature was set at 29°C because this temperature is compatible with a sufficient number of parasitized hosts by *T. cordubensis* and with a great deactivation of symbionts (Pintureau, Bolland, 2001). The three lines were reared until the F51 generation with cycles of temperature including two generations at 23°C (F4, F5; F9, F10; etc.) and three generations at 29°C (F6, F7, F8; F11, F12, F13; etc.). Sex ratios were calculated and selection was performed at each cycle, at the first generation at 23°C (F9, F14, etc.), as described for the F4. Two additional generations at 29°C (F39) or 23°C (F42) prolonged the cycle of selection (6 instead of 5 generations). The selection pressure planned was 10% (10 pro-genies out of 100). In fact, the pressure varied from 9% (F19) to 26% (F34) with a mean of 14% in the high line, and from 11% (F24) to 40% (F40) with a mean of 16% in the low line (tabl. 1).

Results

We were unable to increase or decrease the sex ratio in the selected lines and to discriminate them from the control line (tabl. 1, fig. 1, *A*). The difference between the male proportion observed in the selected lines and in the control line illustrates the absence of efficient selection (tabl. 1, fig. 1, *B*). No correlations of the male proportion with the generation number or regressions against this number were significant (tabl. 2).

Table 1. Variation of the male proportion (total number of males/total number of individuals in all studied progenies) in three *Wolbachia*-infected lines of *T. cordubensis* at 29°C

Таблица 1. Варьирование полового индекса (соотношения количества самцов к общему количество особей во всех изученных поколениях) в 3 линиях *T. cordubensis*, инфицированных *Wolbachia* при 29°C

Generations	Control line		High line				Low line			
	N (1)	Male proportion	N (1)	Male proportion	Male prop./control (2)	Sel. pressure (3)	N (1)	Male proportion	Male prop./control (2)	Sel. pressure (3)
4	74 (1030)	0.12	74 (1030)	0.12	0	0.12	74 (1030)	0.12	0	0.14
9	71 (977)	0.44	68 (925)	0.33	-0.11	0.12	43 (540)	0.33	-0.11	0.23
14	55 (790)	0.07	64 (840)	0.16	0.09	0.16	63 (855)	0.10	0.03	0.16
19	81 (1209)	0.56	69 (983)	0.40	-0.16	0.09	67 (996)	0.46	-0.10	0.15
24	88 (1760)	0.15	71 (1146)	0.27	0.12	0.14	88 (1471)	0.09	-0.06	0.11
29	12 (156)	0.44	17 (208)	0.60	0.16	0.18	63 (829)	0.52	0.08	0.16
34	42 (496)	0.38	38 (566)	0.42	0.04	0.26	56 (723)	0.49	0.11	0.18
40	31 (397)	0.30	45 (615)	0.26	-0.04	0.16	10 (117)	0.33	0.03	0.40
46	60 (921)	0.28	70 (1034)	0.31	0.03	0.14	75 (1074)	0.17	-0.11	0.13
51	79 (1413)	0.25	91 (1923)	0.27	0.02		97 (1917)	0.09	-0.16	

Note. 1 — progeny number and, in brackets, total number of individuals; 2 — male proportion in the high or the low line — male proportion in the control line; 3 — selective pressure, i. e. the number of progenies used to establish the next generation/total number of progenies studied.
Two lines were selected for a high or a low sex ratio, and one line was not selected (control). The selective pressure applied each five or six generations is figured.

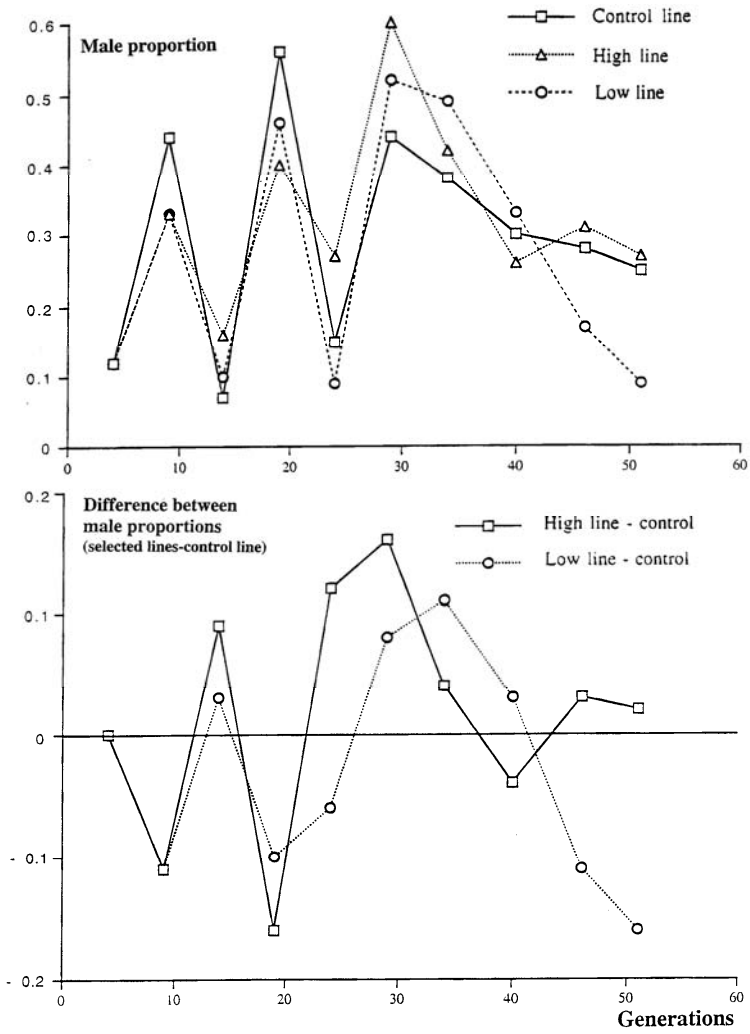

Fig. 1. Male proportion in three lines of *T. cordubensis* selected for a low sex ratio (low line) or a high sex ratio (high line), or not selected (control line), during 51 generations regularly placed at 29°C for three generations (*A*), and difference between male proportions in selected lines and control line (*B*).

Рис. 1. *A* — половой индекс в 3 линиях *T. cordubensis,* выращенных при 29°С в течение 3 поколений; *B* — коэффициент различия между половым индексом в контроле и в линиях с высоким ("high") и низким ("low") половым индексом.

Conclusions and discussion

The unsuccessful selection can be explained by several factors. Heritability of the selected trait could be lower than assumed from the comparison of within- and between-line variances, and/or lower in the strain 1148 than in the strain 802. Moreover, the selection pressure applied, inferior to the planned one, could be insufficient. The frequency of the selection pressure (each 5 or 6 generations) could also be too low.

On the other hand, selection was performed on *Wolbachia* effects on sex ratio and not on the *Wolbachia* density. These effects, only determined by genes of symbionts or also by genes of parasitoids, could show an important epigenetic variability disrupting the selection progress. For instance, females may be mated or not, that especially influences the sex ratio when the female is aposymbiotic. Moreover, the effects could originate from the *Wolbachia* inactivation, which is probably revertible, and not from

Table 2. Correlation with and regression against the generation number (n=10) of the male proportion in three *Wolbachia*-infected lines of *T. cordubensis*, selected or not at 29°C for a high or a low sex ratio

Таблица 2. Корреляция и регрессия количества полового индекса в ряде поколений (n=10) в 3 линиях *T. cordubensis*, помещенных в условия содержания при 29°C

Ratio	Line	Correlation, p>0.05 (2)	Regression (2)
Male proportion	Control	0.159	0.66
	High	0.297	0.40
	Low	0.006	0.99
Male prop./control (1)	High	0.142	0.69
	Low	-0.259	0.47

Note. 1 — Ratio of male proportion in the high or the low line to male proportion in the control line; 2 — proportions were arcsine transformed (for "male prop./control", calculations were performed from differences between two transformed proportions).

the *Wolbachia* elimination. The high temperature resistance of *Wolbachia* could vary in one parasitoid or only in different parasitoids, and combination of the degree of resistance and of the number of resistant symbionts could exist and make the selection difficult. Finally, in the high line, a selected progeny of 90% males can originate from an unmated female infected by some active *Wolbachia* and not from a mated female without *Wolbachia*. To select these latter females, it would be preferable to reject high and low sex ratios and keep progenies with about 30% males.

However that may be, no selection was efficient and the genetic variability of the studied trait could be low. In this case, no advantage for a *Trichogramma* population to be associated to high temperature sensitive *Wolbachia*, allowing bisexual cycles of reproduction in hot regions, could be selected. This would reduce the possibilities of adaptation to the environment (Pintureau et al., 1999) and the interest to be completely infected and thelytokous.

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