Journal of Insect Physiology 120 (2020) 103987

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Role of egg-laying behavior, virulence and local adaptation in a parasitoid's chances of reproducing in a new host

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ARTICLE INFO

Keywords:
Parasitoid
Cotesia typhae
Polydnavirus
Oviposition behavior
Virulence
Biocontrol

ABSTRACT

Understanding the ability of parasitoid insects to succeed in new host populations is a relevant question for biological control and adaptive mechanisms. *Cotesia typhae* is an African parasitoid specialized on the moth *Sesamiae nonagrioides*, also called the Mediterranean corn borer. Two Kenyan strains of *C. typhae* differ in their virulence against a new host population from France. We explored behavioral and physiological hypotheses about this differentiation. *Cotesia* genus belongs to a group of Hymenoptera in which females inject a domesticated virus in their host to overcome its resistance. Since viral particles are injected along with eggs and since the strain with the higher virulence injects more eggs, we hypothesized that virulence could be explained by the quantity of virus injected. To test this assumption, we measured the injected quantities of eggs and viral particles (estimated by viral DNA segments) of each parasitoid strain along several ovipositions, to vary these quantities. Unexpectedly, results showed that virulence against the French host was not correlated to the injected quantities of eggs or viral segments, indicating that virulence differentiation is explained by other causes. The virulence against the respective natural hosts of the two *C. typhae* strains was also measured, and results suggest that local adaptation to a more resistant natural host may explain the pre-adaptation of one strain to the new host population. We also identified a differentiation of oviposition strategy and subsequent offspring number between the parasitoid strains, which is important in a biocontrol perspective.

1. Introduction

Co-evolution, which occurs when species have a reciprocal effect on each other's evolution, is one of the major processes explaining biodiversity (Thompson, 1999). According to Thompson, 1994, parasites have a special place in the study of co-evolution because theirs is the most prevalent lifestyle and they exhibit the most extreme degree of specialization to other species. Among parasites, parasitoids are particular. Indeed, parasitoid females lay their eggs in or on another arthropod to ensure the development of their progeny, which results in the death of the host, whereas most parasites do not kill their host. Hence parasitoids are considered as intermediate between parasites and predators (Godfray, 1994).

Parasitoid reproductive success lies on a wide spectrum of adaptations to their host. Notably, it depends on many traits, including virulence, which indicates the ability of the parasitoid to overcome host immune response. In endoparasitoids, which inject their eggs in their host haemocoele, the main host immune defense with which they must comply is the cellular response called encapsulation (Lavine and Strand, 2002; Salt, 1968). To avoid or suppress encapsulation, parasitoids have developed a large arsenal of weapons. The most studied are venoms produced by eponymous glands, teratocytes formed from the membranes that envelop parasitoid eggs and polydnavirus (Asgari and Rivers, 2011; Beckage and Drezen, 2012; Strand, 2014). The latter is a nice example of mutualistic viruses. The polydnaviridae family groups viruses from multiple origins which were domesticated and integrated in several lineages of Ichneumonoidea. Due to their integration into parasitoid genomes, polydnavirus are vertically transmitted (Fleming and Summers, 1991). They are produced in the form of viral particles containing double-stranded DNA segments, in specialized cells of the calyx, a tissue located in the upper part of the lateral oviducts (Marti, 2003; Wyler and Lanzrein, 2003). These particles are secreted into the lumen of the lateral oviducts and thus injected in the host with the eggs. After oviposition, viral particles infect host cells where virulence genes

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https://doi.org/10.1016/j.jinsphys.2019.103987
Received 29 August 2019; Received in revised form 21 November 2019; Accepted 22 November 2019
Available online 27 November 2019
0022-1910/

carried by DNA segment are expressed. Translated virulence proteins disturb host development and suppress its immune responses, allowing the growth of parasitoid eggs (Beckage and Gelman, 2004; Glatz et al., 2004). Polydnavirus differentiation is involved in the evolution of host range (Herniou et al., 2013), as shown in the *Cotesia* genus member of the Microgastrinae family (Braconidae), which harbors polydnavirus from the genera Bracovirus (Branca et al., 2019, 2011; Jancek et al., 2013).

Like virulence, oviposition strategy can evolve in response to constraints in developmental resources. This is well illustrated by clutch size evolution, a classical topic in ecology, notably in birds (Lack, 1947). Its study is especially relevant in the case of host-parasitoid interactions where host resource is often variable. Many factors such as host size, host quality, host availability, host previous parasitism, parasitoid egg load or experience can influence clutch size, suggesting that females are able to combine information to adjust it (Godfray, 1994; Ikawa and Okabe, 1985; Pexton and Mayhew, 2005; Quicke, 1997; Rosenheim and Rosen, 1991). Beyond the plasticity of this trait, large numbers of injected eggs could also be adaptive, allowing the parasitoid to overcome host resistance by saturating its immune system (Blumberg and Luck, 1990; Kapranas et al., 2012; Rosenheim and Hongkham, 1996; Salt, 1968). The impact of the number of eggs injected on virulence may be addressed. For that purpose, species showing variation in clutch size among individuals is necessary. Such variation was described for *Cotesia typhae* (Fernández-Triana) (Hymenoptera, Braconidae) between two strains from different geographic origins namely Kobodo and Makindu which can interbreed and produce fertile offspring (Benoist et al., 2017).

C. typhae is a gregarious endoparasitoid specialized on caterpillars of *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera, Noctuidae) and was found in Kenya, Tanzania and Ethiopia (Kaiser et al., 2015). This species is prosynovigenic because females emerge with almost all their mature eggs. Thanks to molecular, ecological and morphological analyses, *C. typhae* was recently distinguished from *Cotesia sesamiae*, a generalist species composed of several populations with different host ranges. Local adaptation to *S. nonagrioides* appears to be the main factor responsible for the speciation of *C. typhae* (Branca et al., 2011; Kaiser et al., 2015, 2017a,b).

In the context of biological control, it is necessary to understand the factors that drive the adaptation of the natural enemy to the targeted host. *C. typhae* is one of the sister species of *Cotesia flavipes* (Cameron), which is used worldwide for biological control against maize and sugarcane Lepidoptera stemborers (Postali Parra and Coelho, 2019). The strict specificity of *C. typhae* for *S. nonagrioides* makes it a good potential biocontrol agent against this important crop pest (Cordero et al., 1998; Eizaguirre and Fantinou, 2012), currently in expansion in France (Rousseau, 2009).

In Benoist et al., 2017, the reproductive success of several strains of *C. typhae* from Kenya in a French population of *S. nonagrioides* was investigated. Among the strains used, those from Kobodo and Makindu localities were respectively the most and the least virulent against this new host population. Females from the Kobodo strain injected more eggs, despite an equal initial egg load, and induced a higher expression of two virulence genes in the first host encountered, in comparison to Makindu females. Given that viral particles are injected along with eggs and that Kobodo females inject more eggs at a time, it was hypothesized that they may also inject more viral particles in the host, which may explain the difference of virulence genes expression and thus the difference of virulence.

The first objective of the present work is thus to test if the difference in parasitism success results from a difference in the number of viral particles injected, and if this number varies together with the number of eggs injected. More globally, the second objective is to characterize the reproductive potential of the parasitoid strains in a biocontrol perspective. The last objective is to investigate if the better pre-adaptation of the Kobodo strain to the French host may come from local adaptation to a more resistant host population.

For these purposes, Kobodo and Makindu parasitoid females were allowed to oviposit in three successive hosts of the French population. At each oviposition rank, host acceptance, number of eggs injected, parasitism success (used as an estimate of the parasitoid virulence), offspring number and sex-ratio were measured. The relative quantity of viral segments injected was measured at the two first ovipositions (parasitism success collapsed at the third oviposition). Finally, egg-laying behavior, parasitism success and offspring traits of both parasitoid strains were measured on their African sympatric and allopatric host populations.

2. Materials and methods

2.1. Biological material

The *C. typhae* strains were established from individuals emerging from parasitized larvae collected in two Kenyan field localities: Kobodo (0.679S, 34.412E; West Kenya; collected in 2013) and Makindu (2.278S, 37.825E; South-East Kenya; collected in 2010–2011), which were used to name the strains. They were reared as isofemale lines at the Evolution, Génome, Comportement et Ecologie (EGCE, Gif-sur-Yvette) laboratory from 2015.

Three strains of *S. nonagrioides* were used as host, one from south-west France and two from Kenya. The founder individuals of the two Kenyan strains originated from Kobodo and Makindu, and the founder individuals of the French strain originated from 3 localities in south-west France: Cudos (44.3897 N, −2.2183 W), Biarotte (43.5640 N, 1.2550 W) and Longage (43.3680 N, 1.1926E).

2.2. Insect rearing and parasitism

The larvae of *S. nonagrioides* were reared on an artificial diet (adapted from Overholt et al., 1994) at 26 °C, ca. 60% relative humidity (RH) under a photoperiod of 16:8 (Light:Dark). Three week old larvae from the Makindu host strain were used for the rearing of *C. typhae*, as both parasitoid strains showed similar high reproductive success on this host population. Larvae intended to be parasitized were retrieved and fed for at least 24 h with fresh maize stems before parasitism to increase their acceptance by the *C. typhae* female. On the day of parasitism, the host larvae were placed individually under a 2 cm diameter plastic top with one *C. typhae* female until the ovipositor insertion was observed, insuring that each larva was parasitized only once. Parasitized larvae were then placed in a Petri dish with artificial diet at 27 °C under rearing condition. Once the parasitoid larvae had emerged from the host and spun their cocoons, each parasitoid cocoon mass, corresponding to one progeny, was placed in a disposable plastic box (500 ml) with honey droplets and a water imbibed cotton wool ball under the same rearing conditions. When adults emerged, temperature was decreased to 24 °C and the photoperiod changed to 12:12 to lengthen their life expectancy. In the plastic boxes, siblings were free to mate (mating was not controlled). Females were used for parasitism 1 day after emergence to allow time for mating.

Experiments were conducted in the same conditions as parasitoid rearing. When successive ovipositions were performed, each *C. typhae* female was offered one host larva once a day. This was repeated three times at most (the mean life expectancy of *C. typhae* females is 3 days). Females were one-day old when offered their first host. They were kept individually in a plastic tube (height: 9.5 cm, diameter: 2 cm) with honey droplets and a piece of water-imbibed cotton wool at 21 °C between each host exposure (temperature was decreased to lengthen parasitoid life expectancy).

2.3. Phenotypic traits measure

Several phenotypic traits were measured: host acceptance, number

of eggs injected, parasitism success, offspring number, sex-ratio, and the quantity of viral segments injected (see next section for this last trait).

Host acceptance is the proportion of females that accepted to oviposit within 3 min of host exposure. In order to avoid counting females twice, a female was not considered for further oviposition if it refused to oviposit once. Parasitism success corresponds to the proportion of stung larvae from which parasitoid larvae emerge. To estimate the number of eggs injected at each oviposition rank, females were divided into four groups corresponding to the number of ovipositions they performed (zero to three), and we calculated the difference between the mean numbers of eggs in the ovaries of females of each group. For all dissections, we counted together mature oocytes and the few immature ones. The ovaries dissection protocol is available in Benoist et al., 2017. The offspring number corresponds to the total number of parasitoid larvae that emerged from one host larva (one progeny). The mean offspring number did not include cases without progeny and was estimated only from mixed-sex progenies. Indeed, in haplo-diploid insects like *Cotesia*, unmated females give all-male progeny and in *C. typhae* offspring number varied significantly between mated and unmated females (Benoist et al., 2017). Similarly, sex-ratio was estimated only on mixed-sex progenies. The sex-ratio of *C. typhae* can be determined only at adult stage, so progenies with > 20% of larval mortality (cases where larvae died inside cocoons due to rearing conditions) were not taken into account for the estimation of sex-ratio.

2.4. Comparison of the quantity of viral segments injected in the host

To compare the quantity of viral segments injected in the French host between each treatment (*C. typhae* strain × oviposition rank) we developed a relative qPCR assay. The *S. nonagrioides* larvae were weighed before parasitism. Only larvae with a weight comprised between 260 and 290 mg were kept to ensure that the quantities of hemolymph retrieved were homogeneous between samples. The larvae were dissected 2 h post-parasitism. They were anesthetized by cold exposure (−20 °C) for 20 min. Pro and true legs were cut to collect as much hemolymph as possible. Hemolymph samples were stored at −20 °C until DNA extraction. Before DNA extraction, PBS (Phosphate-Buffered Saline) was added to samples to a final volume of 200 µl. DNA extractions were performed with the NucleoSpin Tissue kit (Macherey-Nagel) following the manufacturer protocol. A RNase (DNase free, Roche) treatment (20 min, 37 °C) was included.

In *Cotesia congregata* and *Cotesia vestalis*, the bracovirus is organized in 35 viral segments (Jancek et al., 2013). A similar number is expected in *C. typhae*. We decided to quantify 15 viral segments, representing about half the putative segment number. The segment-specific primer pairs were designed for qPCR, based on the sequence of *C. sesamiae* Kitale bracovirus segments published in Jancek et al., 2013 and available in GenBank (accession numbers: HF562906–HF562931). The *S. nonagrioides* ribosomal protein gene *rps3* and mitochondrial gene *coI* were used as references for normalization. The primers' efficiencies were calculated using a calibration curve with four 5-fold successive dilution points using a pool of DNA samples and were close to 100%. The primer sequences are presented in Table 1. PCR reactions were run on a CFX96 (Bio-Rad) in 10 µl comprising: 2.4 µl of water, 5 µl of 2X FastStart Universal SYBR Green Master (Roche), 0.3 µM of each primer, and 2 µl of sample DNA (3 µg/µl). A negative control was included for each primer pair and all samples were amplified in duplicate. The amplification protocol was the same for all primer pairs: 10 min of denaturation at 95 °C, followed by 45 cycles of 10 s of denaturation at 95 °C and 30 s of annealing/elongation at 60 °C followed by a melting curve step.

For each primer pair a single peak was observed for all melting curves, indicating specificity and homogeneity of the amplifications. A single threshold mode was used to determine the quantification cycle (C_q) values. If the maximum C_q standard deviation observed between duplicates was higher than 0.4 then the samples were removed.

Table 1
Sequence of the segment-specific and reference genes primers used for qPCR.

Target	Orientation	Sequence
Segment 1	Forward	5′-ACGGAAGCACAGAAAGAACCT-3′
	Reverse	5′-ACCGAAGCTTTCAGGACACA-3′
Segment 2	Forward	5′-GTTTCGGGTGCGTTTCTGTC-3′
	Reverse	5′-ACTTTGCCATCACGCGTTTG-3′
Segment 4	Forward	5′-TCCGCTGCTGTCTCACTTTT-3′
	Reverse	5′-GCCGGGTCCAATTGTTGTTC-3′
Segment 13	Forward	5′-AGAACCGATTTGCTCCCGTT-3′
	Reverse	5′-ACTGCTTTCTAGGTGCTTCAGA-3′
Segment 14	Forward	5′-AGCGTAGTTGATGGCGTTCA-3′
	Reverse	5′-TGCTTCTGAATATGGGCGC-3′
Segment 24	Forward	5′-TCGTCGTCAAGCTTTCGGAA-3′
	Reverse	5′-CCGATCTCACTTGCGAACCT-3′
Segment 26	Forward	5′-TTGATGAGGGAGACGGGGAT-3′
	Reverse	5′-CCCAGGTGTCTTCTATCGGC-3′
Segment 28	Forward	5′-CGCAGGGTATGAAGAGTCCG-3′
	Reverse	5′-ACAACAGAGAGTCGACCAGC-3′
Segment 30	Forward	5′-CCAGGCTGCTGAACCAAAAC-3′
	Reverse	5′-AGCGTCCTGTGGCATAGAAA-3′
Segment 32	Forward	5′-GCGATTTAGCGTGCCAAGAC-3′
	Reverse	5′-TGACGTCAAGCAGCGAAAAG-3′
Segment 33	Forward	5′-CCCACACTCATTTCACCTCCA-3′
	Reverse	5′-GTTCTTACCAGTCGAGCCGG-3′
Segment 35	Forward	5′-TGTACGTCCCAGTAGCACCT-3′
	Reverse	5′-GGAGTGGAAGAATCTGCCCC-3′
COI	Forward	5′-GGAGCCCCAGATATAGCATTTCC-3′
	Reverse	5′-TCATCCTGTTCCAGCCCCAT-3′
RPS3	Forward	5′-GGGAGCTTGCTGAAGATGGC-3′
	Reverse	5′-AGACTGCTCGGGGATGTTGA-3′

The segment numbers correspond to those from *Cotesia sesamiae* Kitale in Jancek et al. (2013).

Relative quantities of viral segments were compared using the $\Delta\Delta C_q$ method [ABI User Bulletin #2 (11–15)].

2.5. Data analysis

All statistical analyses were performed using R Software (R core Team, 2018) with agricolae, fifer and emmeans additional packages. The significance threshold was set at 0.05 p-value. Except for the viral segments relative quantities, all phenotypic traits were analysed using generalized linear model (GLM) to test the effect of each factor (*C. typhae* strain, oviposition/presentation rank, *S. nonagrioides* strain). The error family used in GLM was: binomial for the acceptance and the parasitism success; quasi-poisson for the number of eggs remaining in ovaries and the offspring number; quasi-binomial for the sex-ratio. When a significant effect was observed, a multiple comparison using Tukey's HSD test was performed on the GLM data. The viral segments relative quantities were analysed using the Kruskal-Wallis test, followed by multiple comparison with Holm correction.

3. Results

3.1. Reproductive success in new host population and correlation between oviposition behavior and virulence

To study the reproductive strategy and the mechanisms behind the parasitism success, females from Kobodo and Makindu *C. typhae* strains were allowed to oviposit several times in the French host. Different phenotypic traits were measured at each oviposition rank.

Table 2
French host acceptance by *C. typhae* parasitoid strains.

Presentation rank	*C. typhae* strain	Host acceptance		
		% accepted	n	Statistical test
First host	Kobodo	91.10	123	ab
	Makindu	94.60	111	a
Second host	Kobodo	64.00	114	c
	Makindu	66.10	115	c
Third host	Kobodo	62.30	61	c
	Makindu	78.46	65	b

n = number of *C. typhae* tested females. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following binomial GLM).

3.1.1. Host acceptance

Overall, host acceptance varied significantly with the presentation rank (p-value = 5.947×10^{-14}) but not between *C. typhae* strains (p-value = 0.085). Almost all females (> 90%) accepted to oviposit at the first host presentation (Table 2). At the second host presentation, the acceptance dropped significantly to around 65% for both strains. At the third host presentation a significant difference was observed between the two strains. Indeed, while the host acceptance by Kobodo parasitoid did not differ between second and third presentations, host acceptance by Makindu females increased to 78.5%.

3.1.2. Number of eggs injected

Fecundities of the two *C. typhae* strains were not significantly different: females from Kobodo and Makindu strains produced around 200 oocytes (Table 3). However, the parasitoid strain had a significant effect on the dynamic of egg depletion (p-values = 0.027). At the first oviposition Kobodo females injected around 110 eggs, when Makindu females injected around 72 eggs representing respectively 56% and 34% of their egg load (Table 3). At the second oviposition, the number of eggs injected was reduced by more than half for Kobodo females, whereas only an 11% decrease was observed for Makindu ones. Hence, the number of eggs injected was almost similar for the two strains, around 50–65 eggs. At the third oviposition, Makindu females injected three times more eggs (around 26) than Kobodo ones. This highlighted a difference of oviposition behavior between Kobodo and Makindu females, Kobodo females injecting most eggs in the first host while Makindu females allocated their eggs more equitably along successive ovipositions. Despite these different allocation dynamics, the cumulated predicted number of eggs injected was similar between Kobodo and Makindu: Kobodo = 167.87; Makindu = 162.36.

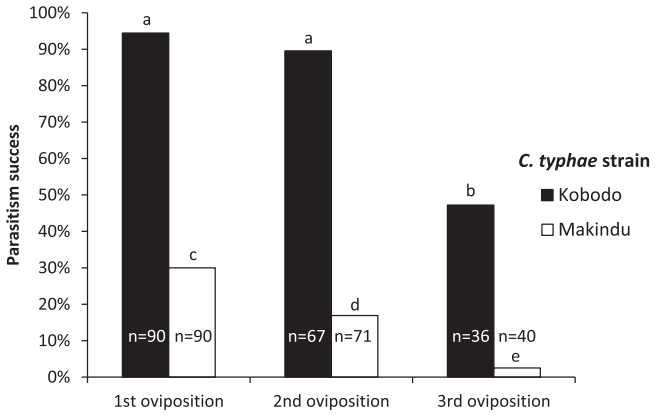

Fig. 1. Parasitism success at each oviposition rank of *C. typhae* parasitoid strains in the French host. Parasitism success corresponds to the proportion of stung larvae from which parasitoid larvae emerged. n = number of host parasitized. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following binomial GLM).

3.1.3. Parasitism success

Cotesia strain and oviposition rank had a significant effect on the parasitism success (respectively p-value < 2×10^{-16} and p-value = 6.744×10^{-8}). A similar pattern was observed at the first and the second oviposition rank: Makindu females had low parasitism success (< 30%), while it was high for Kobodo ones (~90%) (Fig. 1). The parasitism success decreased significantly at the third oviposition, to a level of 50% for Kobodo females and to < 5% for Makindu ones. Globally, the parasitism success decreased along oviposition rank, and the Makindu strain is characterized by its low parasitism success whatever the oviposition rank. Comparison of these results with those from Table 3 showed that the difference of parasitism success between Kobodo and Makindu parasitoids in the French host could not be explained by a difference in the number of eggs they injected: Despite both strains injecting similar number of eggs at the second oviposition rank, their parasitism success is markedly different. The great majority of host larvae that were not successfully parasitized developed normally into pupae, and very few died (3–5% of the total host larvae sample per treatment, as observed in unparasitized host larvae).

3.1.4. Offspring number

The offspring number decreased significantly along the oviposition rank (p-value < 2×10^{-16}) (Fig. 2). It followed more or less the same dynamic as the number of injected eggs, with again a significant effect of *C. typhae* strain (p-value = 7.629×10^{-11}). At the first oviposition, Kobodo females had a higher offspring number than Makindu females.

Table 3
Number of remaining oocytes in ovaries and predicted number of eggs injected per host larva after each oviposition rank of *C. typhae* parasitoid strains in the French host.

Oviposition rank	*C. typhae* strain	Number of oocytes in ovaries				Predicted number of eggs injected per host larva
		mean	se	n	Statistical test	
No oviposition	Kobodo	194.6	± 3.17	116	a	
	Makindu	212.3	± 6.58	44	a	
First oviposition	Kobodo	84.9	± 4.3	46	c	109.7
	Makindu	139.9	± 4.18	71	b	72.4
Second oviposition	Kobodo	34.9	± 2.48	52	e	50.0
	Makindu	75.7	± 5.05	36	c	64.2
Third oviposition	Kobodo	26.7	± 2.36	38	e	8.2
	Makindu	49.9	± 2.97	47	d	25.8

n = number of females dissected; se = standard error. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following quasi-poisson GLM). The number of eggs injected is deduced from the difference between the mean numbers of eggs in the ovaries before oviposition or after one, two or three ovipositions.

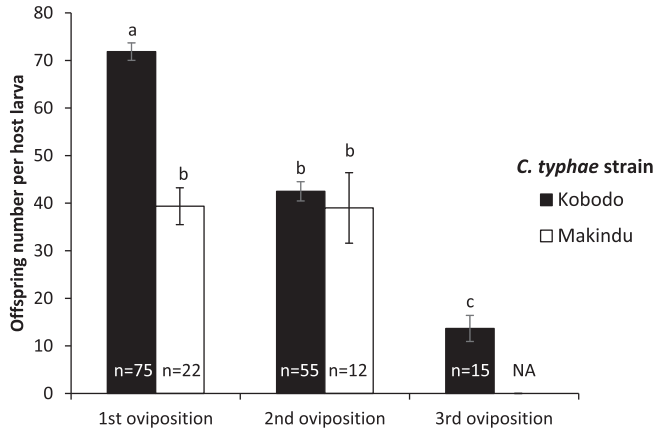

Fig. 2. Offspring number per host larva at each oviposition rank of *C. typhae* parasitoid strains on the French host. n = number of progenies; error bar = standard error; NA = No progeny in this case. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following negative binomial GLM).

The two strains had a similar offspring number, near 40, at the second oviposition. At the third oviposition the offspring number of Kobodo females was low, and that of Makindu could not be estimated due to the lack of progeny development, despite a high rate of host acceptance for oviposition (the only progeny observed in Fig. 1 was discarded because only composed of males).

3.1.5. Sex-ratio

The sex-ratio did not vary significantly with the *C. typhae* strains, while a significant effect of oviposition rank was detected (p-value = 0.037×10^{-5}): The proportion of females in the progeny decreased significantly along ovipositions. The sex-ratios of the progeny were female-biased at the two first ovipositions with about two-thirds to three-quarters being females (Table 4). It notably decreased at the third oviposition rank for Kobodo females, for which a balanced sex-ratio was observed. However, pairwise comparisons were not significant, which was explained by the low number of replicates.

3.1.6. Viral segment quantification

Given that the number of eggs injected in the host caterpillars did not explain the difference of parasitism success between Kobodo and Makindu parasitoids in the French host, a relative quantification of viral DNA segments injected was performed on several segments to see if it could explain this difference.

First of all, as injected quantities of all 15 segments showed a similar pattern (Fig. 3), we may assume that it is also the case for the other non-quantified segments. For the two *C. typhae* strains, the quantity of each viral segment injected decreased significantly by a factor of 2 to 3 between the first and at the second ovipositions. Regarding the difference between the parasitoid strains, it appeared that it was not significant when analysing segments independently, except for the viral segment 17. However, when analysing all segments together, it appeared that during the first oviposition, Kobodo females injected significantly more segments than Makindu females as observed for the number of eggs (Table 3).

To investigate the relation between the quantity of viral segments and the number of eggs injected, we normalized these estimates to the values obtained for Kobodo during the first oviposition (Table 5). Both quantities varied similarly according to parasitoid strain and oviposition rank, but with different amplitude. At the first oviposition, Makindu estimates were lower than Kobodo ones, but the difference in the egg number was more important than the difference in the viral segments. From the first to the second oviposition, the number of segments injected decreased more than the number of eggs; for instance, eggs injected by Kobodo were reduced by 1/2, whereas viral segments were reduced by 2/3.

Most importantly, on average, Makindu females injected more viral segments at the first oviposition than Kobodo females at the second oviposition but parasitism success of Makindu females was lower (Figs. 1 and 3). Hence, neither the viral segments quantity nor the number of eggs injected in the host explained by itself the difference of parasitism success between Kobodo and Makindu females in the French host.

Table 4
Sex-ratio at each oviposition rank of *C. typhae* parasitoid strains in the French host.

Oviposition rank	*C. typhae* strain	Sex-ratio (%females)			
		mean %	se	n	Statistical test
First oviposition	Kobodo	68.41	± 2.15	70	a
	Makindu	76.01	± 3.24	16	a
Second oviposition	Kobodo	62.66	± 3.03	43	a
	Makindu	62.91	± 8.71	8	a
Third oviposition	Kobodo	45.74	± 6.81	8	a
	Makindu	No progeny is this case			

n = number of progenies; se = standard error. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following quasi-binomial GLM).

3.2. Reproductive success in the natural hosts

In all previous experiments parasitism was studied in the French host. Higher success of Kobodo parasitoids in this host may originate from adaptation to a local host with a resistance level comparable to the French host. To test this hypothesis, the parasitism success of the two *C. typhae* strains was measured in their natural hosts from their native localities: Kobodo and Makindu (Table 6). The number of eggs injected, the offspring number and the sex-ratio were also estimated on these hosts.

Kobodo females parasitized all the exposed host strains with a high rate of success (~90%), whereas Makindu ones parasitized only their natural host strain with a high success (Table 6). The parasitism success of Makindu females in the Kobodo host corresponded to that observed in the French one. These two *S. nonagrioides* strains displayed a higher resistance to parasitism by the Makindu parasitoid strain (56 and 59 % of host larvae developed into pupae) than the *S. nonagrioides* strain from Makindu (3.5 % of pupae). Unlike females from the Kobodo strain, females from the Makindu strain had a different offspring number depending on the host strain parasitized. Indeed, they had around 63 offspring in the two Kenyan hosts, while their offspring number was around 40 in the French host. The comparison of the number of eggs injected confirmed that Kobodo females laid more eggs in the first host encountered than Makindu ones whatever the host strain considered. The two parasitoid strains injected fewer eggs in the host from Kobodo than in the other ones. The sex-ratio was neither impacted significantly by the parasitoid strain nor by the host strain and varied from 68.41 ± 2.15 to 76.01 ± 3.24 percent of females.

4. Discussion

Using the host specific *C. typhae* Braconidae as a biological model, the main objectives of this article were to explore the potential link between parasitoid oviposition behavior and virulence against the host, to investigate if higher parasitism success of one parasitoid strain on a new host originated from its local adaptation to a more resistant host, and to characterize the parasitic potential of two parasitoid strains on the new host population targeted for biological control.

Regarding the link between oviposition behavior and virulence, a

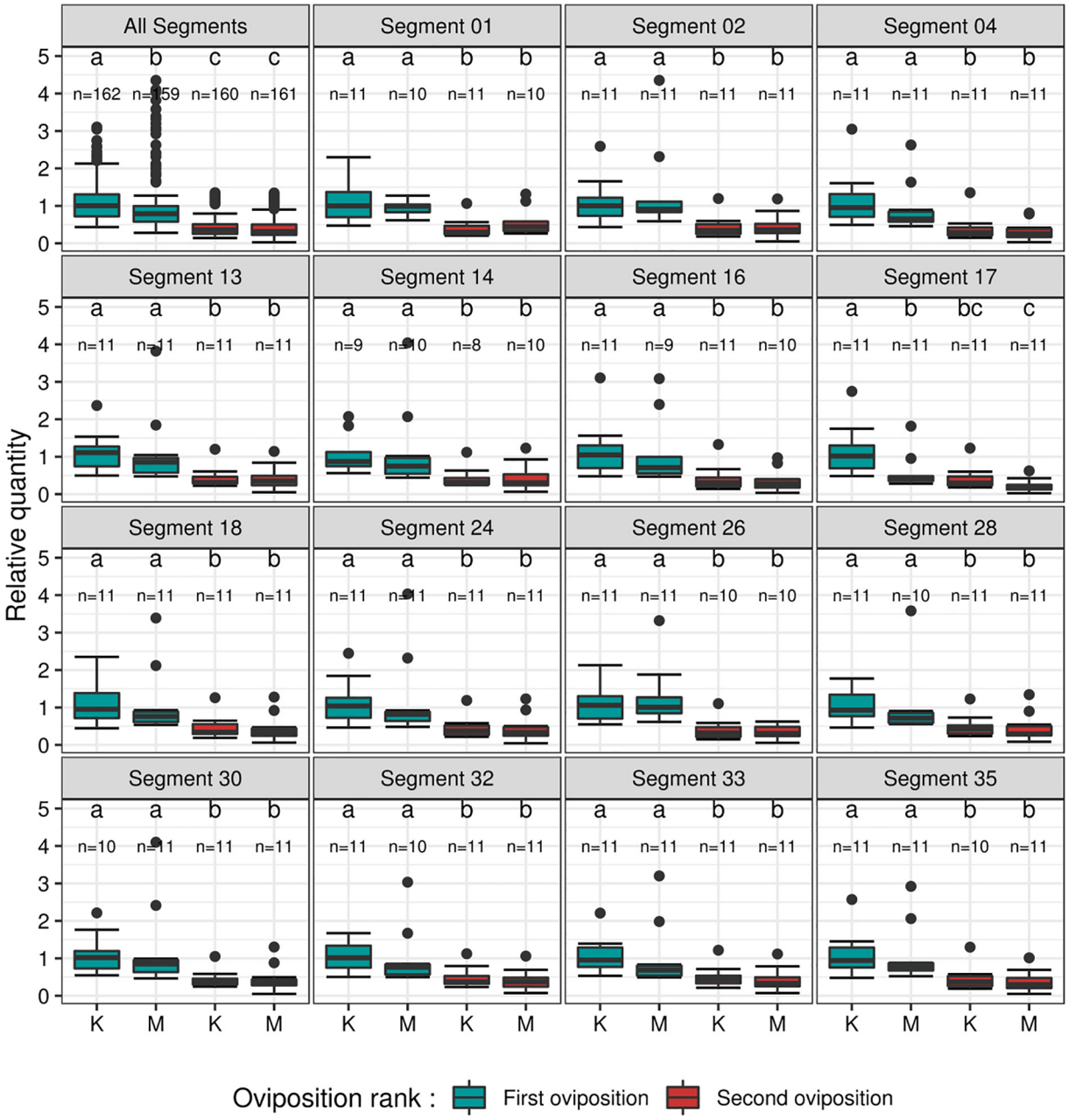

Fig. 3. Relative quantification of the viral DNA segments in the French host hemolymph two hours post-parasitism by *C. typhae* parasitoid strains. K = Kobodo; M = Makindu; n = number of host dissected. "All segments" corresponds to data of all segments pooled. For all boxplots the Kobodo-First oviposition condition is used as reference. Conditions with the same letter are not significantly different (p-value > 0.05, Multiple comparison with Holm correction following the Kruskal-Wallis test). Statistical tests were performed independently for each segment, and for the pool of all segments.

Table 5
Comparison of the injected quantities of segments and eggs in the French host between *C. typhae* parasitoid strains.

Oviposition rank	*C. typhae* strain	Relative quantity of segments injected (standardized median)	Number of oocytes injected (standardized median)
First oviposition	Kobodo	1	1
	Makindu	0.789	0.652
Second oviposition	Kobodo	0.359	0.506
	Makindu	0.313	0.562

For both traits, the medians are given relatively to the Kobodo-First oviposition condition.

previous study on the same species reported differences in the number of eggs injected and in the parasitism success in the French host between Kobodo and Makindu parasitoid strains, when ovipositing once (Benoist et al., 2017). The study showed that the expression of *Cystatin* and *CrV1*, two virulence genes known to inactivate the host immune response (Edson et al., 1981; Glatz et al., 2004), was higher after parasitism by Kobodo, which injected more eggs at a time. Because *C. typhae* females inject eggs and viral particles at the same time in the

Table 6
Comparison of phenotypic traits between *C. typhae* parasitoid strains in the sympatric and allopatric host populations.

Phenotypic trait	*C. typhae* strain	*S. nonagrioides* strain		n	
Parasitism success	Kobodo	**Kobodo**	92.77%	83	a
		Makindu	88.24%	119	a
		French	94.44%	90	a
	Makindu	Kobodo	30.12%	83	b
		Makindu	93.91%	115	a
		French	30.00%	90	b
Offspring number	Kobodo	**Kobodo**	75.96 ± 2.17	70	a
		Makindu	68.91 ± 1.68	88	a
		French	71.85 ± 1.83	75	a
	Makindu	Kobodo	65.79 ± 4.86	24	ab
		Makindu	60.54 ± 1.83	83	b
		French	39.36 ± 3.87	22	c
Predicted number of eggs injected	Kobodo	**Kobodo**	87.66		
		Makindu	108.87		
		French	109.66		
	Makindu	Kobodo	68.1		
		Makindu	78.48		
		French	72.42		

n = sample size; ± = standard error. Conditions in the natural host are in bold. Data in the French host correspond to those presented in previous experiments. Conditions with the same letter are not significantly different (p-value > 0.05, Tukey's HSD test following binomial GLM (Parasitism success) or quasi-poisson GLM (Offspring number)).

host, we expected that the quantities of viral segments injected by Kobodo females would be higher than those injected by Makindu ones, which would explain the higher gene expression and thus higher parasitism success.

However, the present study refutes part of this hypothesis. Indeed, although results showed that the injected quantities of viral segments and eggs varied in the same direction (but not proportionally, Table 5), they showed that the quantity of viral particles injected does not explain the level of virulence because at the second oviposition Kobodo had a much higher success than Makindu despite the similar quantities of eggs and viral segments injected (Figs. 1 and 3).

To explain the difference of virulence between Kobodo and Makindu we assumed a difference of virulence gene expressions (Benoist et al. 2017). This hypothesis cannot be either validated or rejected as yet. If true, it would imply that the virulence gene expression and the quantity of viral segments injected are not correlated, because we showed that Kobodo females have a higher parasitism success at the second oviposition than Makindu females at the first one (Fig. 1), although Kobodo females inject fewer viral segments in this case (Fig. 3). This absence of correlation between the quantity of viral segments injected and virulence gene expression was reported for instance in *Microplitis demolitor* and *Cotesia plutellae* (Beck et al., 2007; Kim and Kumar, 2018) and so is likely in our case. As an alternative to the gene expression hypothesis, the difference of virulence between Kobodo and Makindu strains may result from the efficiency of their virulence proteins, due to possible mutations in the coding sequence. Experiments of gene expression on a higher gene number in larvae parasitized at the second rank, and sequencing of virulence genes of both strains will help validate the right hypothesis.

Viral particle production is initiated in wasp ovaries during late pupal development and can continue throughout the adult stage (Beckage and Drezen, 2012; Marti, 2003; Pasquier-Barre et al., 2002). In several solitary parasitoid species the amount of virus present at adult emergence seems to be enough for all ovipositions during female adult life (Beck et al., 2007; Kim and Sanghoon, 2007; Marti, 2003). To our knowledge the quantity of viral segments injected has never been measured along successive ovipositions in a gregarious parasitoid before this work. For both Kobodo and Makindu females the quantity of viral segments injected decreased at the second oviposition, suggesting a depletion of particle stock (Fig. 3). So in *C. typhae* viral segments production could be insufficient to afford an equal amount of virus for multiple ovipositions, so we can expect that this amount decreases at each new oviposition. At the third oviposition, parasitism success decreased in each experimental combination (Fig. 1). The same phenomenon was observed for the very close species *C. flavipes* that parasites *Diatraea saccharalis* (Scaglia et al., 2005). Even if our results show no correlation between the quantity of viral segments injected and parasitism success, a minimum amount of virus could be necessary to allow successful parasitism, and such a threshold would not be reached at the third oviposition of *C. typhae* due to stock depletion.

Inactivating the host immune system is not the only way parasitoids have to ensure the development of their offspring (reviews in Pennacchio and Strand, 2006; Salt, 1968; Vinson, 1990). For example, in the facultative gregarious endoparasitoid *Metaphycus flavus,* laying multiple eggs decreased the encapsulation rate of the host *Coccus hesperidum* and so increased parasitoid larval survival (Kapranas et al., 2012). Since the number of eggs injected differed between Kobodo and Makindu females (Benoist et al., 2017), an alternative hypothesis to explain the difference of parasitism success in the French host, irrespective of the quantity of viral segments, could be that Kobodo females exhausted the host immune system by laying a large amount of eggs. However, successive ovipositions did not support this assumption because the parasitism success of Kobodo females was higher than that of Makindu ones even with fewer eggs injected (Fig. 1 & Table 3).

Our results also indicated that inactivating the host immune system is not an all or nothing process. Indeed, at the first oviposition, Makindu females got one third less offspring from the French than from its natural host, although they laid almost as many eggs in both hosts. This reveals a lower egg-larval survival rate in the French host. So the difference in offspring number may come from higher encapsulation rate of the eggs and larvae. We observed capsules in this condition, like those described by Gitau et al., 2007, in another noctuid host species parasitized by the sister species *C. sesamiae*, but too few could be observed for reliable counting, due to probable embedding in the host fat body with the same whitish appearance. Thus, even when host larvae allow parasitic development, their immune response is not totally inactivated.

Regarding *C. typhae* behavior, host acceptance by both strains exceeds 90% at the first host exposition, decreases significantly at the second but not at the third (Table 2). Egg load was shown to be an important stimulus for ovipositional activity in several parasitoid species (Collins and Dixon, 1986; Donaldson and Walter, 1988). So, we expected that host acceptance would decrease along ovipositions due to egg depletion. However, no decrease was observed at the third oviposition, suggesting that either the relationship is not linear or other factors drive the motivation to oviposit.

In Benoist et al., 2017 the number of eggs injected was measured only at the first oviposition. On the basis of the observed difference, it was supposed that Kobodo females would inject the majority of their eggs in the first host encountered, while Makindu ones would distribute them more equally. Here, results confirm this hypothesis. Kobodo females lay more than half their eggs in the first host encountered, about 30% in the second and 5% in the third. In Makindu females, the proportions of egg injected in the first and the second host are close (near 30%) and, unlike Kobodo ones, the proportion at the third oviposition remains high (almost 15%). Finally, after the third oviposition, Makindu females have around two times more remaining eggs in their ovaries (> 40 eggs) than Kobodo ones (Table 3), suggesting that the behavioral strain difference would also appear upon a fourth oviposition. Thus, the oviposition behavior of Kobodo females differs from that of Makindu ones, despite close initial fecundities. This difference in egg-laying behavior was not an artifact due to the use of a new host population. Indeed, for all hosts tested Kobodo females injected more eggs at the first oviposition rank than Makindu ones (Table 6). Estimating

the number of opportunities to oviposit in the field could give information about the causes of this differentiation of oviposition behavior. Indeed, the egg allocation strategy of Makindu females will increase their fitness only if they can oviposit multiple times. Thus, we expect that in the field Makindu females have more opportunities to oviposit than Kobodo, which could have resulted in the differentiation of the oviposition behavior by selection. This is in accordance with accessibility to host resource in their natural habitat.

Indeed, Makindu and Kobodo are located in ecologically contrasted regions (Le Rü et al., 2006). The Makindu field location consists in a well limited and dense settlement of *Typha domingensis* localized on the banks of a small stream (B. Le Ru *personal communication)* and surrounded by dry habitat (Somalia-Masai Acacia-Commiphora deciduous bushland and thicket vegetation mosaic, White, 1983). In this habitat *T. domingensis* hosts *S. nonagrioides* as an almost exclusive stemborer (Kaiser et al., 2015). In contrast, the Kobodo region consists of a continuum of wetland located in the mosaic of East African evergreen bushland and secondary Acacia wooded grassland vegetation mosaic, White, 1983), with diversified plant-stemborer species associations (B. Le Ru *personal communication*). So *S. nonagrioides* larvae should be more difficult for the parasitoid to find at the Kobodo locality. Since *C. typhae* are short-lived and thus time-limited, constraints on the probability of finding a host may have favored a higher reproductive investment in the first encountered host.

The reciprocal comparison of the parasitism success of Kobodo and Makindu females in their natural host populations gives information on local host adaptation. While Kobodo females have a high success on both hosts from Kobodo and Makindu, the parasitism success of Makindu females is high only in its sympatric host (Fig. 1 & Table 6). Like the French host, the host from Kobodo resists *C. typhae* females from Makindu but does not resist the local *C. typhae*. This suggests that the French host population has a level of resistance equal to that of the Kobodo Kenyan host population. Thus, local adaptation may explain the ability of Kobodo females to parasite the French host successfully: the higher resistance of the Kobodo host population could have exerted a higher selective pressure on *C. typhae* virulence, which might pre-adapt Kobodo females to the French host.

In southern Europe, *S. nonagrioides* and *Ostrinia nubilalis* are the major pests of maize. Unlike *O. nubilalis* against which *Trichogramma brassicae* are used, no biocontrol agent is available for *S. nonagrioides*. Regarding the potential use of *C. typhae* as a biocontrol agent of *S. nonagrioides* in Europe, this work confirms that Kobodo locality offers an efficient strain against this pest. The performances of this *C. typhae* strain are comparable, for instance, to those of *C. flavipes*, which is used massively in Brazil against the sugarcane pest *Diatraea saccharalis* (Trevisan et al., 2016; Vacari et al., 2012) and are promising for the control of *S. nonagrioides*. The current work shows that Kobodo females could parasite more than one larvae efficiently. Such data are important for mass rearing and for adjusting parasitoid densities for field release.

5. Conclusions

The *C. typhae / S. nonagrioides* system is an attractive system for documenting the mechanisms of adaptation to a host population because it offers differentiation of several reproductive traits between parasitoid strains, and differentiation of resistance between host populations. We addressed mechanisms causing pre-adaptation of a geographical parasitoid strain to an allopatric host pest population targeted for biocontrol. Regarding behavioral and physiological causes, we found that neither the number of eggs injected nor the number of viral particles injected explained the difference of success between strains. Regarding evolutionary causes, our data indicate that pre-adaptation of one strain to the allopatric host population may come from adaptation to its local host. This work also suggests that virulence and oviposition behaviour would have evolved separately, the first in response to the level of host resistance, and the second to environmental variables at play in host availability.

CRediT authorship contribution statement

R. Benoist: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **S. Paquet:** Investigation. **F. Decourcelle:** Investigation. **J. Guez:** Investigation. **R. Jeannette:** Investigation. **P.-A. Calatayud:** Resources, Writing - review & editing. **B. Le Ru:** Resources, Writing - review & editing. **F. Mougel:** Formal analysis, Writing - review & editing. **L. Kaiser:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Odile Giraudier and Sylvie Nortier for insect rearing at Gif, Julius Obonyo for field collection, Claire Capdevielle-Dulac for her advice for the quantification of viral particles, Matthieu Bodet, Aurore Vedel and Nicolas Leblanc for their contribution to the dissections, Taiadjana Fortuna for her help in the experiments, Lionel Saunois, Amandine Dubois and Virginie Héraudet for maize production and Malcolm Eden for linguistic editing of the manuscript. We also thank the anonymous reviewers for their help in improving the manuscript.

Funding

This work was supported by the French National Research Agency (project *Cotebio* ANR-17-CE32-0015), and by the authors' operating grants from IRD, CNRS, and icipe. R. Benoist is funded by the « Ecole doctorale 227 MNHN-UPMC Sciences de la Nature et de l'Homme: évolution et écologie ». Sarah Paquet and Florian Decourcelle were funded respectively by the *Cotebio* project and by the *labex* BASC (project *LutteSesa*). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Asgari, S., Rivers, D.B., 2011. Venom Proteins from Endoparasitoid Wasps and Their Role in Host-Parasite Interactions. Annu. Rev. Entomol. 56, 313–335. https://doi.org/10.1146/annurev-ento-120709-144849.

Beck, M.H., Inman, R.B., Strand, M.R., 2007. Microplitis demolitor bracovirus genome segments vary in abundance and are individually packaged in virions. Virology 359, 179–189. https://doi.org/10.1016/j.virol.2006.09.002.

Beckage, N.E., Drezen, J.-.M. (Eds.), 2012. Parasitoid Viruses: Symbionts and Pathogens, first ed. Elsevier/Academic Press, London; New York.

Beckage, N.E., Gelman, D.B., 2004. Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annu. Rev. Entomol. 49, 299–330. https://doi.org/10.1146/annurev.ento.49.061802.123324.

Benoist, R., Chantre, C., Capdevielle-Dulac, C., Bodet, M., Mougel, F., Calatayud, P.A., Dupas, S., Huguet, E., Jeannette, R., Obonyo, J., Odorico, C., Silvain, J.F., Le Ru, B., Kaiser, L., 2017. Relationship between oviposition, virulence gene expression and parasitism success in Cotesia typhae nov. sp. parasitoid strains. Genetica. https://doi.org/10.1007/s10709-017-9987-5.

Blumberg, D., Luck, R.F., 1990. Differences in the rates of superparasitism between two strains of comperiella bifasciata (howard) (hymenoptera: encyrtidae) parasitizing california red scale (homoptera: diaspididae): an adaptation to circumvent encapsulation? Ann. Entomol. Soc. Am. 83, 591–597. https://doi.org/10.1093/aesa/83.3.591.

Branca, A., Le Ru, B.P., Vavre, F., Silvain, J.-F., Dupas, S., 2011. Intraspecific specialization of the generalist parasitoid *Cotesia sesamiae* revealed by polyDNAvirus polymorphism and associated with different *Wolbachia* infection. Mol. Ecol. 20, 959–971. https://doi.org/10.1111/j.1365-294X.2010.04977.x.

Branca, A., et al., 2019. Relative influence of host, *Wolbachia*, geography and climate on the genetic structure of the sub-saharan parasitic wasp *Cotesia sesamiae*. Front. Ecol. Evol. 7. https://doi.org/10.3389/fevo.2019.00309.

Collins, M.D., Dixon, A.F.G., 1986. The effect of egg depletion on the foraging behaviour of an aphid parasitoid. J. Appl. Entomol. 102, 342–352. https://doi.org/10.1111/j.1439-0418.1986.tb00932.x.

Cordero, A., Butrón Gómez, A.M., Revilla Temiño, P., Malvar Pintos, R.A., Ordás Pérez, A., Velasco Pazos, P., 1998. Population dynamics and life-cycle of corn borers in south Atlantic European coast. Maydica 43, 5–12.

Donaldson, J.S., Walter, G.H., 1988. Effects of egg availability and egg maturity on the ovipositional activity of the parasitic wasp, Coccophagus atratus. Physiol. Entomol. 13, 407–417. https://doi.org/10.1111/j.1365-3032.1988.tb01124.x.

Edson, K.M., Vinson, S.B., Stoltz, D.B., Summers, M.D., 1981. Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid's host. Science 211, 582–583. https://doi.org/10.1126/science.7455695.

Eizaguirre, M., Fantinou, A.A., 2012. Abundance of *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae) on the Edges of the Mediterranean Basin. Psyche J. Entomol. 2012, 1–7. https://doi.org/10.1155/2012/854045.

Fleming, J.G., Summers, M.D., 1991. Polydnavirus DNA is integrated in the DNA of its parasitoid wasp host. Proc. Natl. Acad. Sci. 88, 9770–9774.

Gitau, C.W., Gundersen-Rindal, D., Pedroni, M., Mbugi, P.J., Dupas, S., 2007. Differential expression of the CrV1 haemocyte inactivation-associated polydnavirus gene in the African maize stem borer *Busseola fusca* (Fuller) parasitized by two biotypes of the endoparasitoid *Cotesia sesamiae* (Cameron). J. Insect Physiol. 53, 676–684. https://doi.org/10.1016/j.jinsphys.2007.04.008.

Glatz, R.V., Asgari, S., Schmidt, O., 2004. Evolution of polydnaviruses as insect immune suppressors. Trends Microbiol. 12, 545–554. https://doi.org/10.1016/j.tim.2004.10.004.

Godfray, H.C.J., 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press.

Herniou, E.A., Huguet, E., Theze, J., Bezier, A., Periquet, G., Drezen, J.-M., 2013. When parasitic wasps hijacked viruses: genomic and functional evolution of polydnaviruses. Philos. Trans. R. Soc. B Biol. Sci. 368. https://doi.org/10.1098/rstb.2013.0051.

Ikawa, T., Okabe, H., 1985. Regulation of Egg Number per Host to Maximize the Reproductive Success in the Gregarious Parasitoid, Apanteles glomeratus L. (Hymenoptera : Braconidae). Appl. Entomol. Zool. 20, 331–339. https://doi.org/10.1303/aez.20.331.

Jancek, S., Bézier, A., Gayral, P., Paillusson, C., Kaiser, L., Dupas, S., Le Ru, B.P., Barbe, V., Periquet, G., Drezen, J.-M., Herniou, E.A., 2013. Adaptive selection on bracovirus genomes drives the specialization of *Cotesia* parasitoid wasps. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0064432.

Kaiser, L., Dupas, S., Branca, A., Herniou, E.A., Clarke, C.W., Capdevielle Dulac, C., Obonyo, J., Benoist, R., Gauthier, J., Calatayud, P.A., Silvain, J.F., Le Ru, B.P., 2017a. The *Cotesia sesamiae* story: insight into host-range evolution in a Hymenoptera parasitoid and implication for its use in biological control programs. Genetica. https://doi.org/10.1007/s10709-017-9989-3.

Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R., Calatayud, P.-A., Dupas, S., Herniou, E.A., Jeannette, R., Obonyo, J., Silvain, J.-F., Le Ru, B., 2017b. Systematics and biology of *Cotesia typhae* sp. n. (Hymenoptera, Braconidae, Microgastrinae), a potential biological control agent against the noctuid Mediterranean corn borer Sesamia nonagrioides. ZooKeys 682, 105–136. https://doi.org/10.3897/zookeys.682.13016.

Kaiser, L., Le Ru, B.P., Kaoula, F., Paillusson, C., Capdevielle-Dulac, C., Obonyo, J.O., Herniou, E.A., Jancek, S., Branca, A., Calatayud, P.-A., Silvain, J.-F., Dupas, S., 2015. Ongoing ecological speciation in *Cotesia sesamiae*, a biological control agent of cereal stem borers. Evol. Appl. 8, 807–820. https://doi.org/10.1111/eva.12260.

Kapranas, A., Tena, A., Luck, R.F., 2012. Dynamic virulence in a parasitoid wasp: the influence of clutch size and sequential oviposition on egg encapsulation. Anim. Behav. 83, 833–838. https://doi.org/10.1016/j.anbehav.2012.01.004.

Kim, Y., Kumar, S., 2018. Persistent expression of Cotesia plutellae bracovirus genes in parasitized host Plutella xylostella. PLoS ONE 13, e0200663. https://doi.org/10.1371/journal.pone.0200663.

Kim, Y., Sanghoon, R., 2007. Ultrastructure of *Cotesia plutellae* Bracovirus in its replication at wasp avarian calyx. J. Asia-Pac. Entomol. 10, 357–361. https://doi.org/10.1016/S1226-8615(08)60376-3.

Lack, D., 1947. The significance of clutch-size. Ibis 89, 302–352. https://doi.org/10.1111/j.1474-919X.1947.tb04155.x.

Lavine, M.D., Strand, M.R., 2002. Insect hemocytes and their role in immunity. Insect Biochem. Mol. Biol. 32, 1295–1309.

Le Rü, B.P., Ong'amo, G.O., Moyal, P., Muchugu, E., Ngala, L., Musyoka, B., Abdullah, Z., Matama-Kauma, T., Lada, V.Y., Pallangyo, B., Omwega, C.O., Schulthess, F., Calatayud, P.-A., Silvain, J.-F., 2006. Geographic distribution and host plant ranges of East African noctuid stem borers. Ann. Société Entomol. Fr. NS 42, 353–361. https://doi.org/10.1080/00379271.2006.10697467.

Marti, D., 2003. Ovary development and polydnavirus morphogenesis in the parasitic wasp Chelonus inanitus. I. Ovary morphogenesis, amplification of viral DNA and ecdysteroid titres. J. Gen. Virol. 84, 1141–1150. https://doi.org/10.1099/vir.0.18832-0.

Overholt, W.A., Ochieng, J.O., Lammers, P., Ogedah, K., 1994. Rearing and field release methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borers. Int. J. Trop. Insect Sci. 15, 253–259.

Pasquier-Barre, F., Moreau, A., Huguet, E., Dupuy, C., Drezen, J.-M., Poirié, M., Monteiro, F., 2002. Polydnavirus replication: the EP1 segment of the parasitoid wasp Cotesia congregata is amplified within a larger precursor molecule. J. Gen. Virol. 83, 2035–2045. https://doi.org/10.1099/0022-1317-83-8-2035.

Pennacchio, F., Strand, M.R., 2006. Evolution of developmental strategies in parasitic hymenoptera. Annu. Rev. Entomol. 51, 233–258. https://doi.org/10.1146/annurev.ento.51.110104.151029.

Pexton, J.J., Mayhew, P.J., 2005. Clutch size adjustment, information use and the evolution of gregarious development in parasitoid wasps. Behav. Ecol. Sociobiol. 58, 99–110. https://doi.org/10.1007/s00265-004-0881-7.

Postali Parra, J.R., Coelho, A., 2019. Applied Biological Control in Brazil: From Laboratory Assays to Field Application. J. Insect Sci. 19. https://doi.org/10.1093/jisesa/iey112.

Quicke, D.L., 1997. Parasitic Wasps. Springer, Netherlands.

Core Team, R., 2018. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput, Vienna Austria URL HttpswwwR-Proj.

Rosenheim, J.A., Hongkham, D., 1996. Clutch size in an obligately siblicidal parasitoid wasp. Anim. Behav. 51, 841–852. https://doi.org/10.1006/anbe.1996.0088.

Rosenheim, J.A., Rosen, D., 1991. Foraging and oviposition decisions in the parasitoid Aphytis lingnanensis: distinguishing the influences of egg load and experience. J. Anim. Ecol. 60, 873. https://doi.org/10.2307/5419.

Rousseau, D., 2009. Maïs: la sésamie progresse cap au nord, dans l'air marin et la douceur angevine: progression de la sésamie dans les Pays-de-la-Loire: à gérer dans la région, à méditer ailleurs en France. Phytoma 622–23, 38–41.

Salt, G., 1968. The resistance of insect parasitoids to the defense reactions of their hosts. Biol. Rev. 43, 200–232. https://doi.org/10.1111/j.1469-185X.1968.tb00959.x.

Scaglia, M., Chaud-Netto, J., Brochetto-Braga, M.R., Ceregato, S.A., Gobbi, N., Rodrigues, A., 2005. Oviposition sequence and offspring of mated and virgin females of Cotesia flavipes (Hymenoptera: Braconidae) parasitizing Diatraea saccharalis larvae (Lepidoptera: Crambidae). J. Venom. Anim. Toxins Trop. Dis. 11, 283–298.

Strand, M.R., 2014. Teratocytes and their functions in parasitoids. Curr. Opin. Insect Sci. 6, 68–73. https://doi.org/10.1016/j.cois.2014.09.005.

Thompson, J.N., 1999. Specific hypotheses on the geographic mosaic of coevolution. Am. Nat. 153, S1–S14.

Thompson, J.N., 1994. The Coevolutionary Process. University of Chicago Press, Chicago.

Trevisan, M., De Bortoli, S.A., Vacari, A.M., Laurentis, V.L., Ramalho, D.G., 2016. Quality of the exotic parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) does not show deleterious effects after inbreeding for 10 Generations. PLoS ONE 11. https://doi.org/10.1371/journal.pone.0160898.

Vacari, A.M., De Bortoli, S.A., Borba, D.F., Martins, M.I.E.G., 2012. Quality of Cotesia flavipes (Hymenoptera: Braconidae) reared at different host densities and the estimated cost of its commercial production. Biol. Control 63, 102–106. https://doi.org/10.1016/j.biocontrol.2012.06.009.

Vinson, S.B., 1990. How parasitoids deal with the immune system of their host: An overview. Arch. Insect Biochem. Physiol. 13, 3–27. https://doi.org/10.1002/arch.940130103.

White, F. (Frank), 1983. The vegetation of Africa: a descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa, Natural resources research ; 20. Unesco, Paris.

Wyler, T., Lanzrein, B., 2003. Ovary development and polydnavirus morphogenesis in the parasitic wasp *Chelonus inanitus*. II. Ultrastructural analysis of calyx cell development, virion formation and release. J. Gen. Virol. 84, 1151–1163. https://doi.org/10.1099/vir.0.18830-0.