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(Citation)

Applied Entomology and Zoology, 53(3):291-296

(Issue Date)

2018-08

(Resource Type)

journal article

(Version)

Accepted Manuscript

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<http://dx.doi.org/10.1007/s13355-018-0555-y>

(URL)

<https://hdl.handle.net/20.500.14094/90005136>



Applied Entomology and Zoology (2018) 53:291–296

<https://doi.org/10.1007/s13355-018-0555-y>

Host suitability of the Mediterranean flour moth for rearing *Meteorus pulchricornis* (Hymenoptera: Braconidae), a polyphagous endoparasitoid of pest lepidopteran larvae

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Abstract We investigated the host suitability of the Mediterranean flour moth *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) for a polyphagous koinobiont endoparasitoid *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae), a common natural enemy of various pest lepidopteran larvae. The estimated probability of adult wasp emergence was 80% or higher when eggs were laid in nearly fully-grown larvae of *E. kuehniella* (fresh weight: > 20.0 mg). The body size of emerged adult wasps increased with the initial weight of the host larvae at oviposition. The fresh weight of adult wasps reared on *E. kuehniella* was approximately 60% of that when reared on a natural host *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), and the lifetime fecundity of

wasps reared on *E. kuehniella* was approximately half of that when reared on *S. litura*. *Ephestia kuehniella* was shown to be a positive host candidate for the mass rearing of *M. pulchricornis*, but further investigation is needed to increase the body size of wasps for more practical use of this species as a biocontrol agent.

Keywords Body size • *Ephestia kuehniella* • Fecundity • Longevity • *Spodoptera litura*

Introduction

Efficient and reliable mass rearing of natural enemies is important for successful biological control, especially through augmentation. Parasitoids are generally reared on natural hosts because of the host specificity, but some polyphagous parasitoids, such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), are grown on grain-feeding moths, which can be more cost effective than using the target pest insects (Hoddle and van Driesche 2009; Wang et al. 2013).

Meteorus pulchricornis (Wesmael) (Hymenoptera: Braconidae) is a solitary and koinobiont endoparasitoid of a wide range of free-living lepidopteran caterpillars (Berry and Walker 2004; Fuester et al. 1993; Huddleston 1980; Nishimura et al. 2015; Suzuki and Tanaka 2006, 2007), and is a common natural enemy of *Helicoverpa*, *Mamestra*, *Mythimna* and *Spodoptera* (Lepidoptera: Noctuidae), *Lymantria* (Lymantriidae), *Lasiocampa* (Lasiocampidae), *Lemyra* (Arctiidae), and other pest herbivores in East Asia and Oceania (e.g., Liu and Li 2006, 2008; Maeto 1989; Marsh 1979; Takashino et al. 1998; Walker et al. 2016). This polyphagous parasitoid has been reared on natural hosts and also successfully on a non-natural host, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), for experimental purposes (Askari et al. 1977). Most of the natural hosts of *M. pulchricornis* are plant feeders that eat perishable food; therefore, the rearing of these

species is laborious and tends to be unsanitary even with the use of artificial diets. The wax moth *G. mellonella* seems to be a suitable alternative host for biological experiments but not for mass rearing *M. pulchricornis* due to the high cost of the rearing food.

The Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) has been widely used for mass rearing of *Orius* spp. (Heteroptera: Anthocoridae) and other predatory natural enemies (Cocuzza et al. 1997; Nagai et al. 1998), as well as egg parasitoids (e.g., *Trichogramma* spp.) and larval ectoparasitoids [e.g., *Habrobracon* spp. (Braconidae)] (Borzoui et al. 2016; Smith 1996). This easily reared insect may be a host candidate for mass rearing of *M. pulchricornis*, a highly polyphagous, koinobiont endoparasitoid of lepidopteran larvae.

The host larvae parasitized by *M. pulchricornis* as a koinobiont endoparasitoid remain active and continue feeding and growing in the initial phase of parasitism; finally, the grown host larvae are consumed (Fuester et al. 1993; Harvey et al. 2010). The growth rate of *E. kuehniella* larvae, however, is slow, and the matured larvae are small (Jacob and Cox 1977) when compared with those of *Spodoptera*, *Lymantria* and other natural hosts of *M. pulchricornis*. According to the review of Jervis et al. (2007), the effect of initial host size on the development and progeny size of koinobiont parasitoids varies among cases.

In this study, we evaluated the host suitability of *E. kuehniella* for *M. pulchricornis*, and examined the effects of host body size on the emergence and adult body size of *M. pulchricornis*. We compared the body size, longevity, and lifetime fecundity of adult wasps reared on *E. kuehniella* and on a natural host *Spodoptera litura* (Fabricius) (Noctuidae).

Materials and methods

Insects

The thelytokous strain of *M. pulchricornis* used in the experiments originated from *S. litura* larvae that were collected in Kagawa Prefecture, Japan (Nguyen et al. 2005; KAGAWA_01_U of Abe et al. 2013 and Tsutsui et al. 2014). It had been maintained on *S. litura* larvae that were reared on artificial diets (Insecta LFS, Nosan Corporation, Yokohama). Adult wasps were given with an absorbent cotton ball soaked in 50% honey solution, and kept at 15–20°C under a 16L:8D photoperiod (16 h light and 8 h dark).

A strain of *E. kuehniella* from the Western Region Agricultural Research Center NARO (WARC/NARO) was examined as an alternative host. Larvae were reared on pressed corn under a 16L:8D photoperiod at 25°C.

A strain of *S. litura* from the Sumika Technoservice Corporation, Takarazuka, was reared on Insecta LFS and used as the natural host for comparison.

Experiment 1: To evaluate the size effects of *E. kuehniella* on parasitism of *M. pulchricornis*

Young to nearly fully-grown larvae (fresh weight: 2.0–30.0 mg) of *E. kuehniella* were placed (and made to move using a pair of tweezers) in front of a female adult of *M. pulchricornis* to encourage oviposition (Yamamoto et al. 2009). The oviposited host larvae were reared individually on pressed corn in transparent plastic cases (40 mm diameter × 25 mm height), at 25°C, under a 16L:8D photoperiod for 28 days or until the emergence of mature *M. pulchricornis* larvae. The fresh weight of the host larvae, the parasitoid cocoons, and the emerged adult wasps were measured using a CPA 64 electronic balance (Sartorius AG, Göttingen).

Binominal logistic regression analyses were used to compare the emergence success

of mature parasitoid larvae and adult wasps against the fresh weight of *E. kuehniella* larvae at the time of oviposition. In addition, the fresh weight of *E. kuehniella* larvae immediately before the emergence of *M. pulchricornis* larvae, the developmental period of *M. pulchricornis* from oviposition to larval emergence, and the fresh weight of cocoons and adults of *M. pulchricornis* were analyzed using simple linear regressions against the fresh weight of *E. kuehniella* larvae at oviposition.

Experiment 2: To compare the longevity and lifetime fecundity of *M. pulchricornis* reared on *E. kuehniella* with those on *S. litura*

Nearly fully-grown larvae of *E. kuehniella* (fresh weight: > 20.0 mg) were oviposited by *M. pulchricornis* and reared in the same way as in Experiment 1. Adult wasps were weighed immediately after emergence and individually kept in transparent plastic cases (40 mm diameter × 25 mm height) with an absorbent cotton soaked with 50% honey solution at 25°C, under a 16L:8D photoperiod. The honey solution was continuously provided to the adult wasps as a source of carbohydrates and to prolong the lifespan of the specimens (Wu et al. 2008).

The adult wasps were each provided with 10 individuals of the second or third instar larvae of *S. litura* for oviposition. Every two days, the 10 host larvae were removed and replaced with new larvae until the wasps died. The collected host larvae were dissected to count the number of eggs that had been laid. Because *M. pulchricornis* is a synovigenic endoparasitoid (Fuester et al. 1993), the fecundity was estimated by counting the number of eggs laid in each wasp's lifetime.

Second to third instar larvae (fresh weight: 1.0–6.0 mg) of *S. litura* were oviposited by *M. pulchricornis* and reared on Insecta LFS in transparent plastic cases at 25°C, under a 16L:8D photoperiod. Adult wasps that emerged from the host larvae were treated in the

same manner as outlined above.

The fresh weight of adult wasps at emergence, the longevity, and the lifetime fecundity were compared between those reared on *E. kuehniella* and *S. litura* using the two-tailed median test, referring to Fisher's exact probability value. Simple linear regression analyses were used to assess the longevity and fecundity of adult wasps against the fresh weight at emergence for each host species. Under the assumption of constant linearity throughout the whole range of the fresh weight of adult wasps, an analysis of covariance (ANCOVA) was conducted to test the effects of the host species (*E. kuehniella* vs. *S. litura*), the fresh weight of adult wasps, and their interaction on the longevity and fecundity of wasps.

All of the statistical analyses above were performed using IBM SPSS Statistics version 22 for Windows.

Results

Experiment 1

Out of 100 *E. kuehniella* larvae that were oviposited, 4 host larvae pupated normally, 28 host larvae died, 27 host larvae remained alive without parasitoid emergence on the 28th day after oviposition, and 41 host larvae had a single mature larva of *M. pulchricornis* emerge within 28 days after oviposition. The probability of parasitoid larval emergence increased with the weight of *E. kuehniella* larvae at the time of oviposition (Fig. 1A). Out of the 41 emerged parasitoid larvae, 32 larvae span a cocoon, and 29 adult wasps successfully emerged. The probability of adult emergence increased with the weight of *E. kuehniella* larvae at oviposition (Fig. 1B), indicating that the adult emergence probability of *M. pulchricornis* should be 80% or higher when the fresh weight of the host larvae at oviposition is greater than 20.0 mg.

The fresh weight of *E. kuehniella* larvae immediately before the emergence of the parasitoid larvae was usually less than that at oviposition (Fig. 2A). The developmental period to parasitoid larval emergence decreased with an increase in the fresh weight of *E. kuehniella* larvae at oviposition (Fig. 2B), and reached a constant duration of approximately 10–12 days when the fresh weight of host larvae was over 20.0 mg. The fresh weight of parasitoid cocoons and adult wasps at emergence increased with an increase in the fresh weight of host larvae at oviposition (Fig. 2C).

Experiment 2

The fresh weight of adult wasps reared on *E. kuehniella* was approximately 60% of that of wasps reared on *S. litura*, and this difference was found to be significant (Table 1). The median longevity of wasps reared on *E. kuehniella* appeared to be shorter than that on *S. litura*, while this difference was not significant (Table 1). The lifetime fecundity of wasps reared on *E. kuehniella* was approximately half of that of wasps reared on *S. litura*, and this difference was significant (Table 1).

The linear regression analyses did not indicate a relationship between the longevity of wasps and the fresh weight of those reared on *E. kuehniella* or *S. litura* (Fig. 3A). Positive regressions were found for the lifetime fecundity of wasps and the fresh weight, and were both marginally significant for wasps reared on *E. kuehniella* and *S. litura* (Fig. 3B). The ANCOVA revealed that the effect on lifetime fecundity was not significant for the type of host species (*E. kuehniella* vs. *S. litura*) ($F = 0.143$, $df = 1, 12$, $p = 0.712$), was significant solely for the fresh weight ($F = 8.183$, $df = 1, 12$, $p = 0.014$), and was not significant for their interaction ($F = 0.024$, $df = 1, 12$, $p = 0.880$). The results suggest that the fecundity of wasps simply depends on the body weight regardless of host species; however, this is a hypothetical assessment because the range of fresh weight (covariate)

did not overlap between that of wasps reared on *E. kuehniella* and *S. litura*, as was critically mentioned by Gotelli and Ellison (2004).

Discussion

This study shows that the nearly fully-grown larvae of the Mediterranean flour moth *E. kuehniella* are potential alternative hosts of *M. pulchricornis* for mass rearing practices. By providing larvae of *E. kuehniella* with a fresh weight of over 20.0 mg for oviposition, the possibility of adult emergence is expected to be 80% or higher. However, the period from oviposition to mature larval emergence at 25°C (10–12 days) was somewhat longer than that for wasps reared on *G. mellonella* (ca. 9 days) or *Mythimna separate* (Walker) (Noctuidae) (8–9 days) (Askari et al. 1977; Suzuki and Tanaka 2007). Importantly, the body weight of emerged adult wasps was approximately 60% of that reared on the natural host *S. litura*, and probably due to the small body size, the lifetime fecundity of the wasps (median: 65 eggs) was approximately half of that reared on *S. litura* (median: 139 eggs). These fecundity values are also small when compared to the fecundity of wasps reared on *Lymantria dispar* (L.) (Lymantriidae), which was reported to be 268 eggs on average (Fuester et al. 1993). It is well known that the egg load and lifetime fecundity of parasitoid wasps usually depend on the female body size (Jervis et al. 2007). Small body size may also diminish the dispersal and searching ability of parasitoid wasps (Ellers et al. 1998). One aspect of using *E. kuehniella* in the mass rearing of *M. pulchricornis* that requires attention is the small body size of the emerged wasps.

In Experiment 2, the body weights of the *E. kuehniella* larvae that were provided for oviposition (> 20.0 mg) were much larger than those of the *S. litura* larvae (< 6.0 mg). However, the body weights of adult wasps reared on *E. kuehniella* (median: 1.5 mg) were reversely only approximately 60% of those of wasps reared on *S. litura* (median: 2.6 mg).

Adults of *M. pulchricornis* usually attack young instars of “fast-growing” hosts, such as *S. litura* larvae, and the mature larvae of wasps emerge from enlarged host larvae. As shown by Harvey et al. (2010) for *M. separate* and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the body weight of parasitized host larvae greatly increased until the wasp larvae emerged. The body weight of parasitized *S. litura* larvae was seen to increase up to 30 fold until the emergence of the wasp larvae (S. Nakano unpublished data). In contrast, *E. kuehniella* is a “slow-growing” species, and the body weight of the host larvae was decreased at the time of the emergence of the wasp larvae (Fig. 2A). Our findings revealed that the body size of the parasitoid wasp *M. pulchricornis* is limited by the body size of *E. kuehniella* larvae at oviposition. To obtain *M. pulchricornis* wasps that are more effective biocontrol agents, we suggest that enlargement of *E. kuehniella* larvae for parasitoid oviposition by improving rearing conditions or selective breeding will be necessary.

Another aspect requiring a solution is how to make wasps oviposit on *E. kuehniella* larvae, which are usually hidden among grains. As a parasitoid of free-living caterpillars exposed on plant leaves, adult *M. pulchricornis* attack moving hosts using visual cues (Yamamoto et al. 2009). In the experiments of the present study, oviposition was induced by moving a host larva with tweezers in front of the wasps; however, this methodology is not practical for the mass rearing of wasps without an ingenious contrivance to make the host larvae move autonomously to encourage the wasps to attack. In addition, the defensive behaviors of *E. kuehniella* larvae against wasp oviposition are not known, but may need to be considered (Zhou et al. 2017).

In conclusion, the Mediterranean flour moth may serve as a positive host candidate for the mass rearing of *M. pulchricornis*; however, further studies are needed to provide wasps of sufficient quality for practical use of this species as a biocontrol agent.

Acknowledgments We thank Madoka Nakai and Yasuhisa Kunimi for providing the wasp strain that was examined in this study. This research was, in part, supported by Japan Society for the Promotion of Science KAKENHI Grant Number 17K19268.

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Table 1 The fresh weight at emergence, longevity, and lifetime fecundity of <i>M. pulchricornis</i> adults reared on <i>E. kuehniella</i> and <i>S. litura</i>										
	<i>E. kuehniella</i>					<i>S. litura</i>				
	Median	upper quartile	lower quartile	<i>n</i>		Median	upper quartile	lower quartile	<i>n</i>	<i>p</i> value ^a
Wet weight (mg)	1.5	1.35	1.8	9		2.6	2.5	2.8	7	0.001
Longevity (days)	36	23	45	9		44	42	50	7	0.315
Fecundity (eggs)	65	24	106	9		139	106	169	7	0.035

^a Fisher's exact probability (two-tailed) for the median test

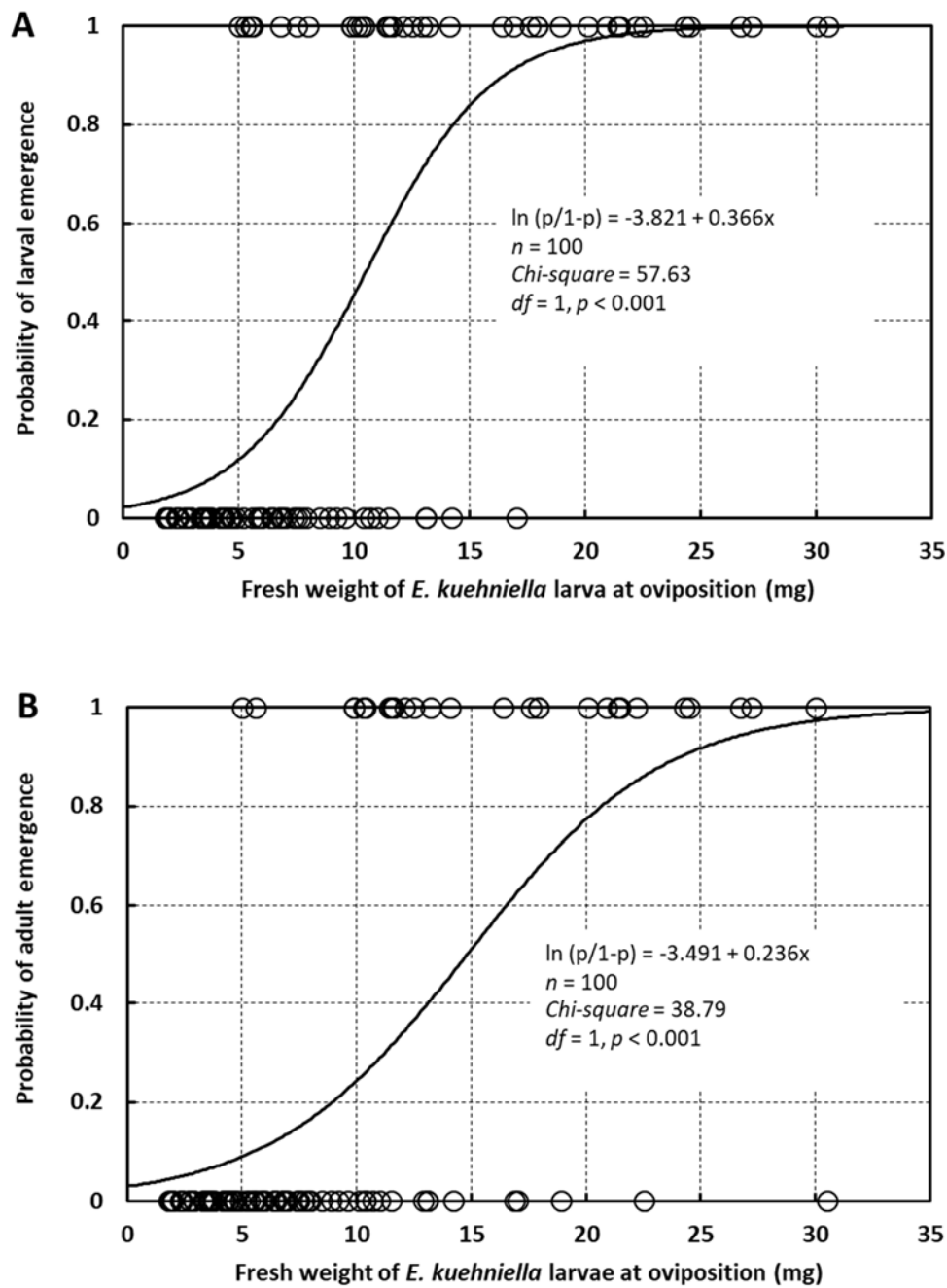
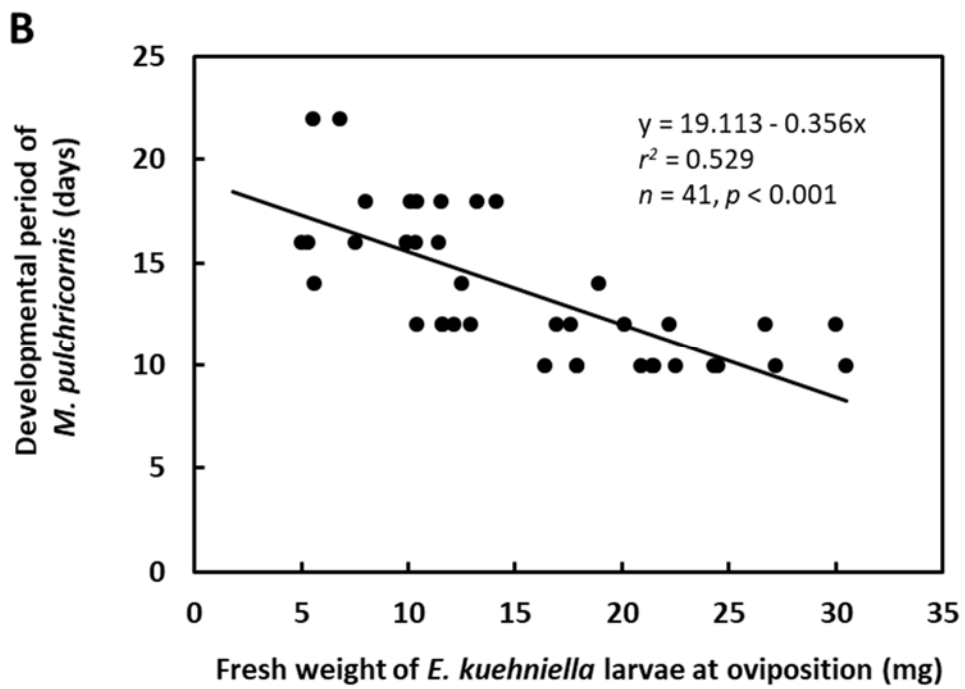
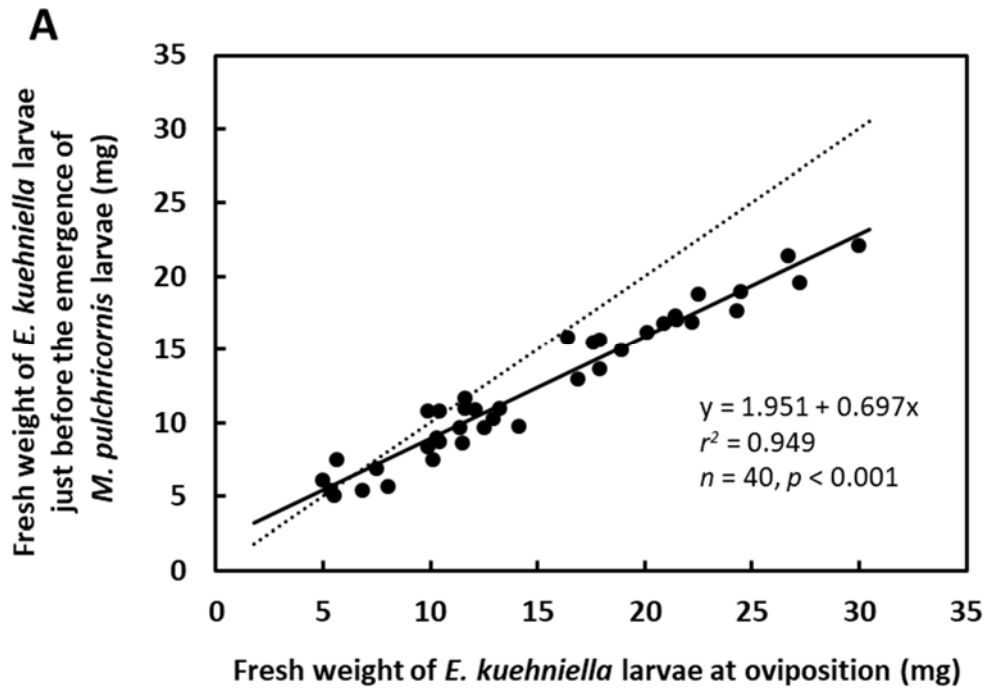


Fig. 1 Logistic regressions of mature larval emergence (**a**) and adult emergence (**b**) of *Meteorus pulchricornis* against the fresh weight of *Ephestia kuehniella* larvae at oviposition. Each point represents an individual of *M. pulchricornis* that either successfully emerged (1) or did not (0)



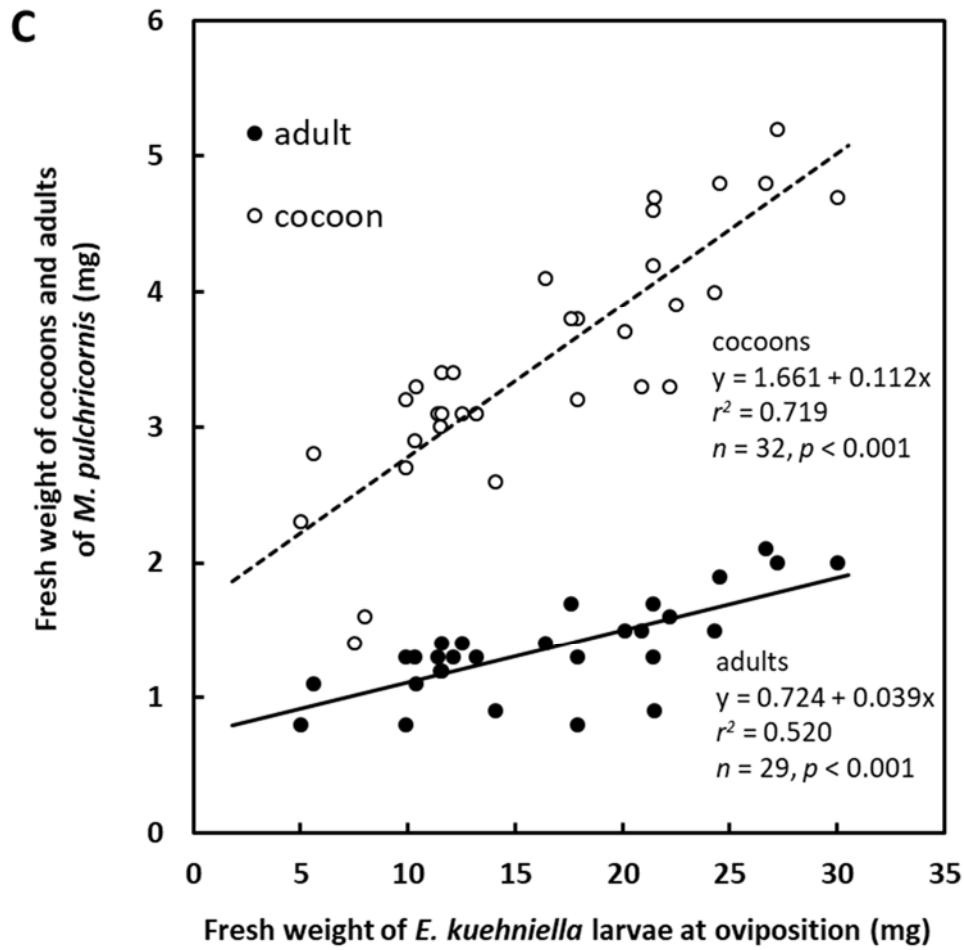


Fig. 2 Linear regressions of the fresh weight of *Ephestia kuehniella* larvae immediately before the emergence of *Meteorus pulchricornis* larvae (**a**), the developmental time of *M. pulchricornis* from oviposition to larval emergence (**b**), and the fresh weight of cocoons or adults of *M. pulchricornis* (**c**), all against the fresh weight of *E. kuehniella* larvae at oviposition. Dots in (**a**) indicate the line of equality

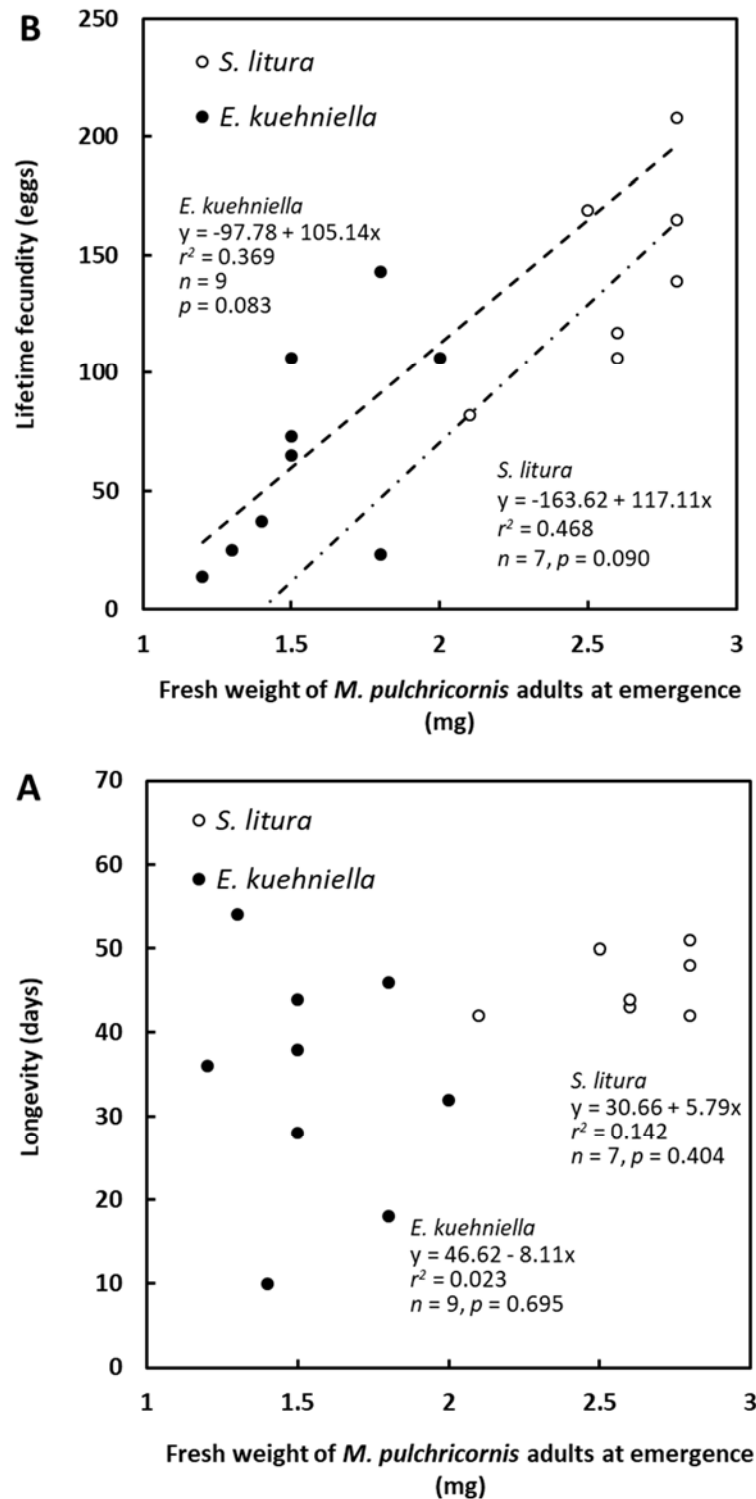


Fig. 3 Relationships of the longevity (a) and the lifetime fecundity (b) of *Meteorus pulchricornis* adults reared on *Ephestia kuehniella* or *Spodoptera litura* to the fresh weight of adults at the time of emergence