Effects of Experience on Subsequent Feeding or Oviposition Preference in *Musca domestica* (Diptera: Muscidae) and *Chrysomya megacephala* (Diptera: Calliphoridae)[1]

Zheng-Yan Wang and Jian-Chu Mo[2]

Urban Entomology Research Center, Institute of Insect Sciences, College of Agriculture and Biotechnology, Zhejiang University, Zhejiang 310029 P. R. China

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Abstract Objectives of this research were to evaluate the effect of larval experience on feeding preference of larvae and the effect of larval and pupal experience on oviposition preference of females in *Musca domestica* L. and *Chrysomya megacephala* F. Dietary experience from hatch to test significantly influenced feeding preference of the second-instar larvae in *M. domestica* ($P < 0.01$), but did not in *C. megacephala*. The larval dietary and pupal experience did not change the oviposition preference of *C. megacephala*, and experienced gravid females laid all of their eggs on pork muscle. Larval feeding regimen had no effect on oviposition preference of subsequent females in *M. domestica*. However, the pupal experience in *M. domestica* significantly influenced the oviposition preference of subsequent females, and *M. domestica* females from pupae matured on wheat bran laid significantly more eggs on wheat bran than those that matured on pork muscle, or filter paper whether pupae were washed or not ($P < 0.01$). These results demonstrated that host-selection behavior in *M. domestica* was shaped by preimaginal experience according to the chemical legacy hypothesis.

Key Words *Musca domestica*, *Chrysomya megacephala*, experience, host-selection

In the urban environment, putrescible garbage is the main habitat of flies. This resource is ephemeral, usually persisting for no more than one generation, and its components change rapidly. Therefore, flies would change their feeding and oviposition sites under the condition of unstable resources. Learning, defined as a behavioral change resulting from experience, may serve a function in the habitat selection process of flies. It is adaptive to adjust to variable conditions and increases the probability of fly survival. Experiences of hosts can modify insect feeding (Akhtar et al. 2003, de Boer 2004, Chow et al. 2005) and oviposition preferences (Rietdorf and Steidle 2002, Akhtar and Isman 2003, Chow et al. 2005, Olsson et al. 2006).

Acceptance of a host is governed either by stimulation signals or by the absence of deterrents (Chow et al. 2005). Thus, the diet-induced food preference in insects may be the result of one or a combination of several different physiological processes, such as habituation to feeding deterrents and sensitization to dietary chemicals (de Boer 2004). Preimaginal conditioning in holometabolous insects gives rise to two controversial theories. One is Hopkins' Host-Selection Principle, which is described as larval memory of the feeding substrate that is stored in the central nervous system and

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[2]Address inquiries (email: mojianchu@zju.edu.cn).

transferred through the influential metamorphism to the adult stage (van Emden et al. 1996); the other is chemical legacy hypothesis, which suggests that traces of chemicals obtained during the larval stage in the hemolymph of the insect or on the outside of the pupa might modify the perception and behavior of the adults (Corbet 1985).

The housefly, *Musca domestica* L., and the oriental latrine fly, *Chrysomya megacephala* F., are common synanthropic fly species in most areas in the world. Acceptable host substrates are wheat bran and pork muscle for the housefly and fish meal and pork muscle for the blowfly in the laboratory. Fly eggs are laid readily on these substrates, and hatching larvae feed and develop into normal pupae and adults. Here, we investigated effects of dietary experience on feeding preference of subsequent larvae and larval dietary and pupal experience on oviposition preference of subsequent adult females in the housefly and the blowfly.

Materials and Methods

Source of flies. Laboratory colonies of the housefly and the blowfly were used for all evaluations. The housefly colony was obtained from the Shanghai Institute of Entomology, Chinese Academy of Sciences, in 2001, and the blowfly colony originated from adults captured in Hangzhou, Zhejiang province, China, in 2005. Colonies were reared under laboratory conditions for more than 10 generations. Adult flies were kept in a nylon mesh cage (35 × 35 × 35 cm) and supplied with water and brown sugar. Wet wheat bran was the oviposition substrate and larval food for the housefly, and minced fresh pork muscle was the oviposition substrate and larval food for the blowfly. The colonies were maintained at and all experiments were performed at 25°C, 14L:10D, and 60% RH.

Feeding preference. The choice box for larval food preference tests was composed of 3 plastic boxes (8 × 10 × 4 cm) connected to each other with 3-cm long polyethylene tubes (1.8 cm diam). The bottoms of the boxes contained wet filter paper, and the tops were screened. Ten g minced fresh pork muscle was placed in the left box, and wet wheat bran (65% moisture, housefly) or fish meal (45% moisture, blowfly) was placed in the right box; 20 second-instar housefly or blowfly larvae were placed in the middle box. Prior to the test, housefly larvae were reared on wheat bran or pork muscle, and blowfly larvae were reared on pork muscle or fish meal. The larvae were rinsed with distilled water before choice tests. After 10 h during the photophase, the number of larvae in each box was recorded. The test was replicated 15 times.

Oviposition preference. Housefly larvae were reared on wheat bran or pork muscle, and blowfly larvae were reared on fish meal or pork muscle till prepupation to provide different larval food experiences. To provide different pupal experiences, housefly prepupae with the larval food experience of wheat bran or pork muscle were matured on wheat bran, dried pork muscle or filter paper, and blowfly prepupae with the larval food experience of fish meal or pork muscle matured on fish meal, dried pork muscle or filter paper in a plastic box till adult emergence; a portion of housefly and blowfly pupae matured on filter paper were thoroughly cleaned with absorbent cotton in distilled water immediately after pupation, and then supplied with clean filter paper. Newly-emerged adults were exposed to pupation substrates in the box <12 h after emergence.

Five pairs of 10-15 day-old adults with different preimaginal experiences were placed in a cage with 10 g wet wheat bran (65% moisture) and fresh pork muscle (housefly) or with 10 g wet fish meal (45% moisture) and fresh pork muscle (blowfly). The substrates were changed daily, and the number of fly eggs on each substrate was recorded daily for 4 d. Each test was replicated 10 times.

Statistical analysis. The data from the larval choice test were analyzed with a paired *t*-test (Tang and Feng 2002) within a treatment and with a one-way ANOVA (Tang and Feng 2002) between treatments. The effect of the pupal experience on the substrate acceptance of housefly females with the same larval dietary experience in oviposition tests was analyzed with a one-way ANOVA and Duncan test (Tang and Feng 2002). Because there were significant differences among oviposition responses of housefly females with different preimagnial experiences ($P < 0.01$), we set larval food (wheat bran and pork muscle) and pupal experience (wheat bran, pork muscle, filter paper, filter paper with pupal water-washing) as fixed factors and analyzed these data with a two-way ANOVA and Duncan test (Tang and Feng 2002). All statistical analyses were executed using the DPS© software (Tang and Feng 2002).

Results

Effect of feeding experience on larval behavior. Dietary experience had a significant effect on feeding preference of housefly larvae ($F = 12.8$; $df = 1, 28$; $P < 0.01$). When reared with wheat bran, subsequent second-instar larvae showed no preference to wheat bran or pork muscle in the choice test ($t = 0.5$, $df = 14$, $P = 0.65$). However, second-instar housefly larvae with the dietary experience of pork muscle showed significant preference to pork muscle ($t = 4.1$, $df = 14$, $P < 0.01$). Dietary experience had no clear effect on the feeding preference of subsequent second-instar blowfly larvae ($F = 0.4$; $df = 1, 28$; $P = 0.53$), which always demonstrated greater preference to fish meal ($t = 6.2, 14.2$; $df = 14$; $P < 0.01$; Table 1).

Effect of preimaginal experience on adult oviposition preference. Regardless of larval dietary and pupal experience, subsequent blowfly adult females laid all of their eggs on pork muscle. However, there was a significant effect of preimaginal experience on housefly oviposition preference ($F = 4.7$; $df = 7, 72$; $P < 0.01$). When housefly larvae were reared on wheat bran, subsequent females with the pupal experience on wheat bran and filter paper laid significantly more eggs on wheat bran than

Table 1. Effect of dietary experience on food preference of larvae (Mean ± SE, *n* = 15)

Larval species	Dietary experience	Percentage of larvae on a nutrition substrate (%)		
		Wheat bran	Pork muscle	Fish meal
Musca domestica	Wheat bran	49.58 ± 5.60aA*	44.34 ± 5.74aA	—
	Pork muscle	21.76 ± 5.42aB	70.06 ± 6.40bB	—
Chrysomya megacephla	Fish meal	—	13.00 ± 5.34aA	77.67 ± 5.41bA
	Pork muscle	—	8.92 ± 2.22aA	81.85 ± 3.68bA

* Means of the same species within each column followed by the same capital letter are not significantly different with a one-way ANOVA and means of the same species within each row followed by the same small letter are not significantly different with a paired *t*-test ($P > 0.05$).

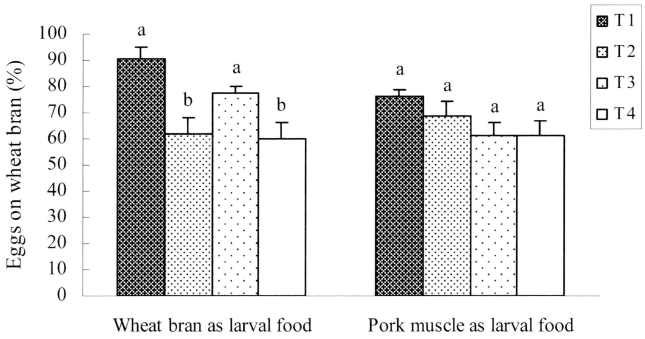

Fig. 1. Oviposition preference of *M domestica* (Means ± SE, *n* = 10) with the larval experience of wheat bran or pork muscle, and the pupal experience of wheat bran (T1), pork muscle (T2), filter paper (T3), or filter paper but pupae were washed with distilled water (T4). Means with the same letter within the same cluster are not significantly different with a one-way ANOVA and Duncan test ($P > 0.05$).

those with the pupal experience on pork muscle, or filter paper with water washing of pupae ($F = 7.6$; $df = 3, 36$; $P < 0.01$). When housefly larvae were reared on pork muscle, there was no significant difference among the oviposition responses of females with different pupal experiences ($F = 2.2$; $df = 3, 36$; $P > 0.05$; Fig. 1).

Data from oviposition responses of housefly adult females with different preimaginal experiences were pooled for two-way ANOVA. Larval dietary experience did not influence the oviposition preference of resulting housefly adult females, but pupal experience significantly impacted preference (interaction of larval food*pupal experience: $F = 2.6$; $df = 3, 72$; $P > 0.05$; larval experience: $F = 2.1$; $df = 1, 72$; $P > 0.05$; pupal experience: $F = 7.7$, $df = 3, 72$; $P < 0.01$). Housefly adult females with the pupal experience on wheat bran laid significantly more eggs on wheat bran than those with any other pupal experience.

Discussion

A change in food preference may be due primarily to habituation to feeding deterrents, sensitization to dietary chemicals, or both (de Boer 2004). When housefly larvae were reared with wheat bran, pork muscle did not deter the experienced second-instar larvae in choice tests. Therefore, deterrents for the housefly are apparently absent in pork muscle and, thus, the change of feeding preference to pork muscle demonstrated by housefly larvae with dietary experience with pork muscle might be induced by stimulants in the pork muscle that are either ingested or contacted on the body.

The blowfly colony had been reared with pork muscle for generations, but larvae showed great preference to fish meal in spite of this previous dietary experience. Fish meal is a more inferior food for blowfly than pork muscle and, as a result, larvae reared with fish meal exhibited longer larval development time, lower preadult survival, and smaller adult body size than those reared with pork muscle (unpubl. data). As attraction of the stimulant chemicals to insect chemoreceptors is always dose-dependent and restricted within a narrow range of concentration (Chapman 2003), maybe there are some common stimulants, not relating with nutritional demand of blowfly larvae in pork muscle and fish meal, and the optimal concentrations of these required chemical signals in fish meal make it more attractive to the larvae.

Pronounced changes in the nervous system are associated with metamorphosis and there are seldom convincing evidences for preimaginal conditioning of holometabolous insects (Barron 2001). However, it was shown for *Drosophila* that interneurones of the olfactory pathway persist through metamorphosis and could be involved in memory retention over metamorphoses (Tissot and Stocker 2000). Studies with dipteran flies demonstrate that memory transfer from the larval to the adult stage is possible (Tully et al. 1994, Ray 1999). Nevertheless, the oviposition behavior was more significantly influenced by pupal experience than by larval experience in houseflies substantiated the chemical legacy hypothesis to some extent. Minute amount of chemicals on the insect body surface are assumed to influence the insect's preferences (Corbet 1985).

Barron and Corbet (1999) demonstrated that the effects of chemical legacy of menthol might be reduced or eliminated by washing the pupae. When housefly larvae were reared with wheat bran, subsequent adult females with the pupal experience on filter paper with water washing of pupae laid significantly less eggs on wheat bran than those with pupal experience only on filter paper. This suggests that chemicals involved in the oviposition preference might be water-soluble. These chemicals usually are highly related to taste chemosensory of insects and need to be studied further.

Blowflies have been reared on pork muscle diet in our laboratory for more than 10 generations, so a selection effect or genetic predisposition could shape the host-selection process. This genetically-determined response might have masked any effects of larval dietary and pupal experience on the host-selection behaviors (Rietdorf and Steidle 2002, Olsson et al. 2006). In our test, adult blowfly females showed an innate response to pork muscle, independent of preimaginal exposures. However, houseflies did not exhibit the innate feeding or oviposition response, which might result from the wide larval food spectrum of this polyphagous species.

The probability that an insect will feed or oviposit on a particular host will depend on the acceptability of the host to the insect. This will be influenced by both innate tendencies and prior experience. These mechanisms could act independently or synergistically, increasing or decreasing the probability of acceptance (Akhtar and Isman 2003). Our test indicated that the feeding habit or oviposition preference of a generalist (housefly) has stronger plasticity than that of a specialist (blowfly), and housefly larvae and adult females possess the ability to learn the features of a new suitable food resource. This might allow the cosmopolitan distribution of the synanthropic housefly in the world, especially in the areas in which the available nutrition resources are ephemeral and their constitution is successively changing.

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