Effect of Nymphal Diet on Adult Predation Behavior in *Orius majusculus* (Heteroptera: Anthocoridae)

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ABSTRACT   The predatory bug *Orius majusculus* (Reuter) was reared on 2 different diets during the nymphal stages. The 1st group was exclusively offered eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), a standard diet for *O. majusculus* production. The 2nd group was exclusively offered 4th instars of the pea aphid, *Acyrtosiphon pisum* (Harris). Subsequently, adult predatory behavior in experimental arenas containing *A. pisum* was recorded using 2 video cameras. One camera permitted observation of the predator's contact with the prey, where the 2nd camera viewed the arena from above to record the path taken by *O. majusculus* adults before and after contact with prey. When *O. majusculus* were reared on aphids, adult bugs successfully located and consumed 55% of experimental prey and continued prey search behavior after each aphid meal. *O. majusculus* adults that had no experience of aphid predation as nymphs, did not prey on aphids in the experimental arena. The mean walking speed of this group of predators increased from 5.9 ± 1.2 mm/s to 9.8 ± 0.7 mm/s after contact or detection of prey, indicating that predators rapidly moved away from unfamiliar prey. Moreover, for egg-reared *O. majusculus*, all contacts between aphid and predator were lateral, along the side of the prey and were effectively repelled by an aphid kicking response. In contrast, 83% of attacks by aphid-reared *O. majusculus* were directed at the head or posterior abdomen for which the prey could not defend themselves adequately. When egg-reared *O. majusculus* were exposed to novel aphid prey for 1–8 d, the frequency of aphid attack increased significantly. We conclude that the standard diet used for rearing *O. majusculus* may adversely affect the efficiency of this predator as an agent of biological control.

KEY WORDS *Orius majusculus*, aphid, biological control, lepidopteran eggs, immature diet, feeding behavior

Orius majusculus (REUTER) is a polyphagous predator that attacks aphids such as *Phylloxerina salicis* (Litch) (Strawinski 1964) and other aphid species infesting fruit trees (Carayon and Steffan 1959). *O. majusculus* may also attack lepidopteran eggs and mites (Pericart 1972, pp. 160–190). This generalist predator is currently used as an agent for the biological control of the thrips *Frankliniella occidentalis* (Pergande) (Fischer et al. 1992). Mass-rearing techniques for *O. majusculus* were developed by Alauzet et al. (1992) using a nymphal diet of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. Adult *O. majusculus* are released for biological control because juvenile stages suffer high mortality and show low predation rates and low capabilities for dispersal; even releases of the adult stage can result in insufficient pest control (Grasselly 1994).

Two types of insect predator search behavior have been defined (Jander 1975, Bell 1991). The 1st, ranging search, involves rapid, long-distance movements to locate prey items (Bell 1985). The 2nd, local search,

involves slow sinuous search paths for precise prey location. This duality in prey search behavior was reported by Ferran et al. (1994) who observed ranging search in coccinelids before contact with aphid secretions followed by intensive local search behavior after contact with such stimuli. Ferran et al. (1997) also reported an effect of rearing on the coccinelid *Harmonia axyridis* (Pallas); larval coccinelids reared on the aphid *Acyrtosiphon pisum* (Harris) showed a clear response to aphid residues, whereas conspecifics reared on lepidopteran eggs did not.

In the current study, we initially attempted to determine the effect of 2 types of nymphal diet, lepidopteran eggs or aphids, on the adult search and predation behavior of the generalist predatory bug *O. majusculus.* Subsequently, we studied changes in the behavior of *O. majusculus* adults that had been reared on lepidopteran eggs when offered aphid prey over time. We attempted to relate these laboratory observations to the possible efficiency of this predator as an agent of biological control.

Materials and Methods

Rearing. The *O. majusculus* used in this study were from a laboratory culture maintained following techniques described by Alauzet et al. (1992). The insects

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were reared in plastic cylindrical containers (10 cm diameter by 7.6 cm tall, 600-cm^3 capacity) placed over a water reservoir to maintain a constant 80% RH. A temperature of 18°C and a photoperiod of 16:8 (L:D) h were used to avoid ovarial diapause (Alauzet et al. 1992). Each cylinder contained a geranium (*Pelargonium peltatum* Struck) leaf for *Orius* oviposition, a shelter of folded paper, and food (lepidopteran eggs), which was scattered inside the container every 2 d.

In the case of the experimentally reared insects, the 1st group of 100 *O. majusculus* were fed exclusively during the nymphal stages on eggs of *Ephestia kuniella* Zeller that had been reared on maize, *Zea mays* L., grain. The 2nd group, equal in size, were offered living pea aphids, *Acyrthosiphon pisum* (Harris), that had been reared on pea, *Pisum sativum* L., plants. These 2 groups are referred to hereafter as egg-reared and aphid-reared, respectively.

After passing through 5 nymphal instars, preadults from each group were placed in separate cylinders without food. Nine hours after adult emergence, *O. majusculus* showed prey searching behavior (Hénaut 1997) and were used for experimental observations.

Experimental Procedures. The experimental arena was composed of a cylinder of plain white paper (20 cm tall and 20 cm in diameter) with a circular paper base. Previous studies indicate that *O. majusculus* was capable of visual detection of prey in any part of an arena of this size (Hénaut 1997). In the center of the arena a living, a 4th-instar pea aphid was secured in place by sticking 1 leg to the paper with Super glue (type SG-HC, Via-Chem, St Laurent, Quebec, Canada). Pea aphids of this stage were not larger than the *O. majusculus* predators.

A single *O. majusculus* selected at random from the rearing group was carefully introduced 5 cm away from the aphid. A pair of video cameras (model CCD-TR 2000, Sony, Tokyo) attached to recorders were used to register the movement of the predator from 2 angles: one above the arena to determine the search path of the predator, and the other with a higher magnification lens focused close-up on the aphid to record antennal contact and details of prey attack by *O. majusculus.* Each observation lasted a maximum of 10 min or was stopped when the predator reached the limits of the experimental arena and made contact with the edge of the arena. The arena paper and prey were changed after each observation. In total, 40 *O. majusculus* adults were observed from each rearing group.

Analysis of Video Recordings. The frequency of contact of *O. majusculus* to the aphid prey was compared by a chi-square test. The video images were digitized on a personal computer (1 frame every 40 milliseconds) and the position of the predator in each frame was considered as point coordinates on an X–Y grid. The image digitalization program used was developed by A. Ferran in the Laboratory of Invertebrate Research of the Institut National de la Recherche Agronomique of Antibes in France. *O. majusculus* behavior was classified into 2 phases: before and after evaluation or contact with the prey. The shortest distance observed between predator and prey was taken as the moment of evaluation of the prey as *O. majusculus* adults showed a highly characteristic behavior associated with detection and evaluation of prey at close proximity, that involved stopping briefly, close to prey, with the antennae extended directly toward the prey, indicative of prey evaluation in the absence of contact (Hénaut 1997). Subsequent observations confirmed that it was appropriate to consider predators that evaluated prey with or without contact as a single group. This technique had been successfully used previously to analyze the search paths of coccinelid larvae (Ferran et al. 1997).

Trajectory indices were calculated by taking into account the displacement velocity (mm/s) and the number of momentary halts during walking for each second of the total search time. The straightness of the search path was calculated as the direct distance between the start point and the finish point divided by the distance of the actual search path taken: values approach 0 for highly sinuous paths and 1 for very straight search paths. In this way, information was obtained on the searching pattern of *O. majusculus* from each rearing group, before or after contact or closest approach to the prey. The means of the trajectory indices calculated for each phase of *O. majusculus* behavior were compared for the 2 rearing groups using a Mann–Whitney *U* test ($P = 0.05$).

Prey contact for each rearing group was analyzed by classifying the site of 1st contact between *O. majusculus* and the aphid prey. Two classes of contact were defined frontal/posterior (1st contact made with head or hindmost part of abdomen of the aphid) or lateral (1st contact made on the side of the thorax or along the side of the aphid abdomen). These results were then subjected to a chi-square test.

Changes in Aphid Predation Over Time. Following the procedures of the previous experiment, an additional study was performed using *O. majusculus* adults from the egg-reared group. Randomly selected adult predators were confined with aphid prey ($\approx$50 aphids replaced every 48 h) in the rearing cylinders for 1 d ($n = 45$), 2 d ($n = 45$), 3 d ($n = 50$), or 8 d ($n = 33$). The *Orius* were then observed using the experimental arena described above. Comparisons of predatory behavior (number of predator–prey physical contacts and consumption of prey) were made in relation to egg-reared *O. majusculus* adults ($n = 40$) that had no previous experience of aphid prey (controls).

Results

Prey Contact and Attack. Twenty of the 40 egg-reared *O. majusculus* were observed to make contact with the aphid prey, compared with 100% of the aphid-reared predators ($\chi^2 = 26.6$, df = 1, $P < 0.001$). None of the egg-reared *Orius* attacked or consumed the aphid, whereas 55% of aphid-reared *O. majusculus* consumed the prey during the observation period.

Search Path. For aphid-reared *Orius*, there was no significant difference between the mean ± SE search velocity before (7.3 ± 1.2 mm/s) or after (7.7 ± 1.4
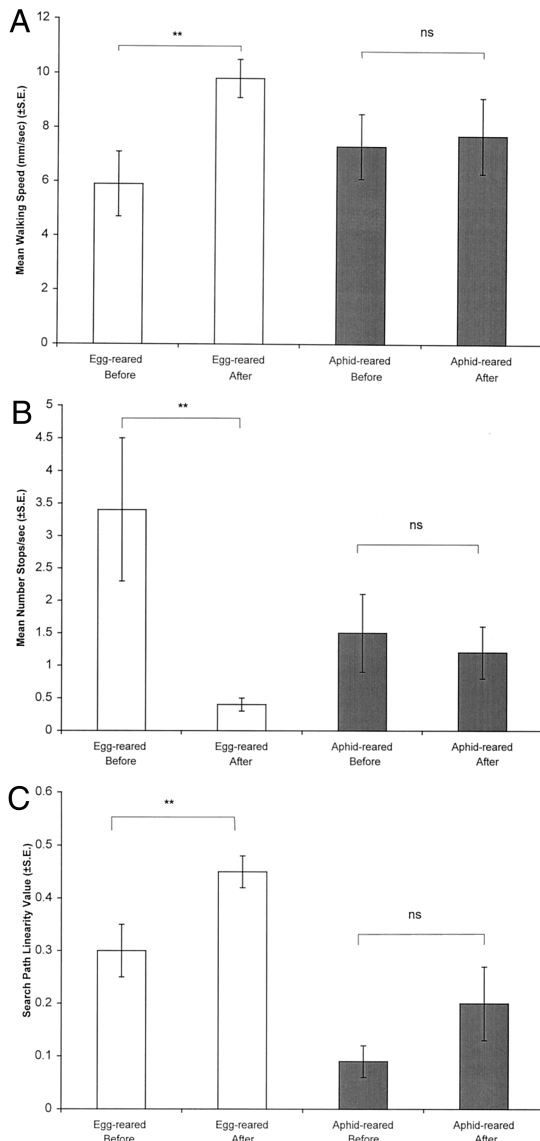
Fig. 1. Comparison of search path characteristics for egg-reared and aphid–reared *O. majusculus* before or after contact or closest approach to the experimental aphid prey, analyzed by Mann–Whitney *U* test, (**, *P* < 0.01; ns, not significant at *P* > 0.05). (A) Mean walking speed. (B) Mean number of stops per 2nd. (C) Linearity value of search path (values approaching zero indicate highly sinuous search, values approaching unity indicate a straight search path). In all cases, vertical bars indicate standard error of the mean.

mm/s) contact or attack of the prey (Fig. 1A). In contrast, egg-reared predators showed a significant increase in their mean ± SE walking speed from 5.9 ± 1.2 mm/s before contact or evaluation of the aphid prey, compared with 9.8 ± 0.7 mm/s after contact ($U = 0.00$, $P < 0.01$) (Fig. 1A).

The number of halts in the search path of aphid-reared *O. majusculus* was not affected by contact with

the prey (Fig. 1B), whereas for egg-reared predators, the mean ± SE frequency of pauses fell from 3.4 ± 1.1 to 0.4 ± 0.1 pauses per second after prey contact ($U = 628$, $P < 0.01$).

The straightness of the search path of aphid-reared *O. majusculus* did not change significantly after prey contact. The search path of egg-reared *O. majusculus* was initially straighter than that of aphid-reared conspecifics with a mean ± SE linearity factor of 0.30 ± 0.05 and this became even straighter (0.45 ± 0.03) after contact with the unfamiliar aphid prey ($U = 5$, $P < 0.01$) (Fig. 1C).

Attack Behavior. Of the 20 contacts that occurred between aphid- and egg-reared *O. majusculus*, all were lateral contacts along the side of the aphid. In all cases these potential attacks were repelled effectively by a reflex kicking action of the aphid's legs. In contrast, of the 40 observed aphid contacts by aphid-reared *O. majusculus*, 83% were directed at the head or posterior abdomen and just 17% were lateral contacts ($\chi^2 = 20.48$, df = 1, $P < 0.001$). Frontal-posterior attacks resulted in consumption of the prey in 55% of cases.

Changes in Aphid Predation Over Time. The proportion of egg-reared *O. majusculus* that made direct contact with the aphid was very low (1/40) for predators that had no aphid experience. After 1 d of exposure to aphids, 13.3% of *O. majusculus* made contact with this prey and this increased steadily from 22.2% after 2 d of exposure, 26.0% after 3 d of exposure to to 36.3% after 8 d of exposure to *A. pisum* ($\chi^2 = 13.01$, df = 4, $P = 0.011$).

Similarly, the frequency of prey consumption increased from zero for the inexperienced *O. majusculus*, 4.4% for the bugs exposed to the aphid for 1 d, 6.6% after 2 d of exposure, 22.0% after 3 d of exposure, and 24.4% after 8 d of exposure to aphids ($\chi^2 = 17.15$, df = 4, $P = 0.0018$).

Discussion

This study demonstrated some important aspects of the predatory behavior of *O. majusculus*. First, this predator failed to investigate or consume a prey type different from that which it had experienced as a nymph. After approach to the unfamiliar aphid prey, the predator's search path became more linear and walking speed increased, which may reflect an escape or avoidance response by the predator.

There appeared to be a degree of learning in egg-reared *O. majusculus* because after 1–8 d exposure to the novel prey type, the frequency of predation increased from 4.4% to >20%. This, however, was lower than the frequency of attacks seen in *O. majusculus* that had been raised on aphids, for which the frequency of attack was 55%.

Attack of the prey was not effective in egg-reared *O. majusculus* because all contacts were made along the side of the aphid's body where the predator could be easily dislodged by the aphid's kicking response. This defense is ineffective against direct frontal or rear attacks, which is a strategy that adult *O. majusculus* apparently develop as nymphs.

This study demonstrates that for a generalist insect predator, experience gained as an immature affects decisions and behavior in the adult stage. Such patterns have previously been reported for phytophagous insects and parasitoids (Papaj and Prokopy 1989, Kerguelen and Carde 1996). The ability of social insects to learn to recognize and capture novel prey is well recognized (Beckers et al. 1994, Dejean et al. 1990, Dejean et al. (1993). In the case of parasitoids, chemical cues have been demonstrated to be of key importance in prey (host) recognition for both generalist and specialist parasitoids (Kerguelen and Carde 1996).

The stimuli by which *O. majusculus* oriented to prey habitat during ranging search has also been studied, and visual cues, mediated by previous experience, appear to be particularly important (Hénaut et al. 1999). Moreover, at close range, *O. majusculus* reared during the nymphal stages with prey associated with a substrate of a particular color oriented to substrates of the same color as adults (Hénaut et al. 1999). During local search, *O. majusculus* depends on previous experience to be effective in attacking and subduing prey. McAuslane et al. (1991) observed a similar phenomenon for parasitoids.

Ferran et al. (1997) suggested that a predator may be far more efficient in the field if experienced in dealing with the target prey. It appears that a rearing environment for immature predators that is deficient in prey-related stimuli or prey diversity may impede the effectiveness of this predator. A degree of caution is needed in relating the findings of our laboratory study using artificial arenas to field situations and it is evident that these results require confirmation in large scale field studies. Our study does however indicate the importance that diets used for insect rearing can have on predator performance and highlights the role of behavioral studies in quality control of mass-reared predators released for biological control.

Acknowledgments

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