Journal of Insect Behavior, Vol. 16, No. 6, November 2003 (© 2003)

Short Communication

Coccinellid Learning During Capture of Alternative Prey

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Accepted July 16, 2003; revised September 11, 2003

KEY WORDS: Coccinellidae; *Anisolemnia*; behavioral flexibility; learning; predation.

INTRODUCTION

In animals, environmental unpredictability favors behavioral flexibility over genetically determined behavior and, thereby, favors conditioning, i.e., behavioral learning or experience-related changes in behavior that lead to increased fitness. Many insects are capable of associative learning, particularly operant conditioning, which involves modifications in behavior through rewards (sugar water for bees) or punishment (caterpillars avoiding feeding on certain plants after suffering an adverse response). Also, many cases of insect learning correspond to habituation, a nonassociative type of conditioning in which individual responses decrease when a stimulus is repeated. For example, an insect alerted to a possibly dangerous situation adjusts its behavior with slower and more efficient movements (Stephens, 1993; Leahey, 1998; Völkl, 2001).

Most coccinellid beetles are specialized predators, with a mixed diet composed of "essential prey" capable of supporting larval development and adult reproduction and "alternative prey" that only enable adults to survive when essential prey are lacking (Evans *et al.*, 1999). The larvae of the coccinellid *Anisolemnia tetrasticta* (Fairmaire), specialized in the capture of nymphs of *Libyaspis* sp. (Heteroptera; Plataspidae), always detect prey by contact. In response, the *Libyaspis* nymphs cower, so that the margins of

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their laterally hypertrophied tergites come into contact with the substrate. These morphological and behavioral defenses are foiled by morphological and behavioral adaptations of the coccinellid, as the larvae slide their hypertrophied forelegs under the nymphs, lift them, and bite them on ventral surfaces. *Anisolemnia* larvae occasionally prey on another plataspid, *Caternaultiella rugosa* (Schouteden), when during periods of proliferation, some nymphs develop outside the pavilions where they are generally attended by ants. The *Caternaultiella* nymphs, devoid of morphological defenses, try to escape upon contact with the ladybird larvae that adopt the previously described behavior, or directly grasp then bite them, showing a behavioral plasticity when confronted with this alternative prey (Dejean *et al.*, 2002).

We hypothesize that this behavioral plasticity could be coupled with learning, with a tendency for the larvae to display the sequence "grasp then bite" the *Caternaultiella* nymphs more frequently after several encounters. Learning in coccinellids, little documented, has only been recorded in *Stethorus* concerning shifts in prey preferences after conditioning and in *Adalia* larvae that learn to avoid distasteful and poisonous aphids (Houk, 1986; Dixon, 2000).

MATERIALS AND METHODS

This study was undertaken in southern Cameroon, where *Caternaultiella* individuals develop in carton pavilions built by the formicine ant *Camponotus brutus* (F.) at the base of *Bridelia* spp. (Euphorbiaceae) trees. *Anisolemnia tetrasticta* females lay their eggs on branches where *Libyaspis* proliferates. Late-instar larvae, which prey on nymphs of a wide range of sizes, also explore parts of the trees other than those occupied by *Libyaspis*, thus enabling them to encounter the *Caternaultiella* at the base of the trunks (Dejean *et al.*, 2000, 2002).

We tested 28 last-instar *Anisolemnia* larvae originating from *Bridelia* that did not shelter *Caternaultiella*, so that they were confronted with this alternative prey for the first time when we began the experiments. We chose last instars because ladybird larvae become more voracious and successful at capturing prey as they increase in size (Dixon, 2000). The ladybird larvae were bred in plastic petri dishes (8 cm in diameter) whose bottom was covered with a piece of *Bridelia* bark, keepin g prey and predator from being influenced by the artificial substrate. For the experiments, which began 2 days after the ladybird larvae were installed, every 3 days we introduced one last-instar *Caternaultiella* nymph into each petri dish. We noted each time if, during their attempts at capture, the ladybird larvae slid their forelegs under the prey or if they grasped the prey before biting. If not captured 10 min after their introduction (several attempts at capture can occur), the prey were removed, and we considered this situation as "prey escaped." Four possible situations can therefore occur: Either the ladybird larva slides its forelegs under the prey and successfully captures it (SC) or the prey escapes (SE); the ladybird larva grasps and captures the prey (GC) or attempts to grasp the prey, which escapes (GE).

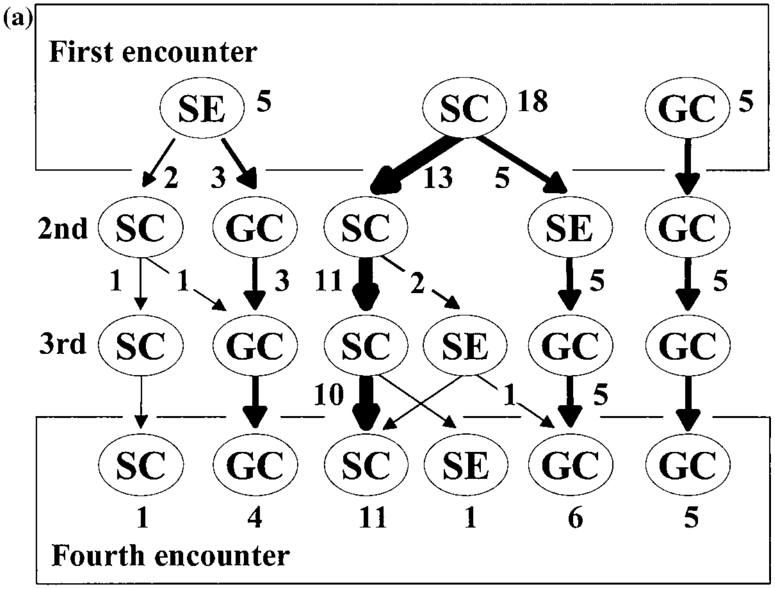
To test if a kind of learning occurred during the four series of encounters with *Caternaultiella* nymphs, we compared the observed transition dyads (SC/→/SE, for example) with the null hypothesis of independent events (absence of learning), resulting in a theoretical distribution of these dyads. For comparisons, the probability of occurrence of each dyad was calculated for both the observed and the theoretical transition dyads. We also compared (a) the observed and theoretically expected frequencies of dyads (chi-square test) and (b) the number of cases when a ladybird larva "slid its forelegs" or "grasped" after SC, SE, and GC (Fisher's exact test; Statistica 5.0 software). Appropriate probabilities were adjusted for the number of simultaneous tests, using the sequential Bonferroni procedure (Rice, 1989).

Voucher specimens of ants, plataspids, and coccinellids were deposited and identified at the Museum of Natural History, London.

RESULTS AND DISCUSSION

From the first encounter 5 of 28 *Anisolemnia* larvae attacked last-instar *Caternaultiella* nymphs by grasping them with their hypertrophied forelegs (18%; Fig. 1), confirming the interindividual variability and behavioral flexibility previously noted during a field study (Dejean *et al.*, 2002). The behavior "grasp the prey" was always followed by a successful capture (GC), resulting in no occurrence of GE. This was not the case for "slide the forelegs under the prey," which resulted in successful captures (SC) after one or several attempts in less than 10 min or only unsuccessful attempts (SE).

The comparison of the observed transition dyads with their theoretical homologues resulted in a significant difference (see Fig. 1b), giving weight to the following comparisons between observed and theoretical values. After a ladybird larvae performed "grasp the prey" once, it always used this behavior in the encounters which followed, resulting in a probability value of 1 for GC to occur when GC was previously performed (observed dyads GC → GC), while the expected value based on the null hypothesis is $P = 0.378$ (Fig. 1). After an unsuccessful attempt at capture (SE), the ladybirds tended to grasp the prey, with $P = 0.75$ for the observed dyad SE → GC, significantly



(b)

Observed values				Expected values			
→	**SE**	**SC**	**GC**	→	**SE**	**SC**	**GC**
SE	0	3	9	**SE**	1.509	6.616	4.875
SC	8	36	1	**SC**	6.616	29.009	21.375
GC	0	0	27	**GC**	4.875	21.375	15.750

(c)

Probabilities				Probabilities			
→	**SE**	**SC**	**GC**	→	**SE**	**SC**	**GC**
SE	0	0.25	0.75	**SE**	0.116	0.509	0.375
SC	0.18	0.80	0.02	**SC**	0.116	0.509	0.375
GC	0	0	1	**GC**	0.116	0.509	0.375

Fig. 1. (a) Comparison of four successive attacks of last-instar nymphs of the plataspid heteroptera *Caternaultiella rugosa* by 28 last-instar larvae of the coccinellid beetle *Anisolemnia tetrasticta*. SE—the ladybird slid its forelegs under the prey, which escaped; SC—the ladybird slid its forelegs under the prey and captured it; GC—the ladybird grasped the prey and captured it. For comparisons, a table of observed and theoretically expected values of transitions of dyads and their corresponding probabilities is included. (b) Comparisons of the observed and expected values: dl = 4; $\chi^2 = 62.66$; $P < 0.001$. (c) Comparisons of the occurrences of the behaviors "slide the forelegs under the prey" (i.e., sum of SE plus SC) and "grasp the prey," after the three situations (Fisher's exact test and sequential Bonferroni procedure): SE versus SC, $P < 0.001$; SE versus GC, $P < 0.05$; SC versus GC, $P < 0.001$.

higher than the expected value ($P = 0.375$). For the same reasons we noted significant differences comparing the occurrences of the behaviors "slide the forelegs under the prey" versus "grasp the prey" when the ladybird larvae previously performed SE, SC, or GC (Fig. 1c).

These comparisons support our hypothesis that repeated experience with alternative prey will result in *Anisolemnia* larvae more frequently grasping these prey than using the more complex behavior adapted to foil the defenses of their essential prey. As this conditioning was associated with immediate, successful attacks, it seems to reinforce the subsequent learning to grasp alternative prey. Moreover, unsuccessful individuals, hungry in comparison with the others, also had a tendency to perform grasping during the next encounter with alternative prey. The parsimony of behavioral acts when grasping alternative prey, which saves extra effort, could also play a role as a reinforcing factor. Grasping, which occurred from the first encounter with a *Caternaultiella* for certain *Anisolemnia* larvae, is therefore "genetically determined," with an interindividual gradation for performing this act: from the first encounter, after learning, or maybe never. This diversity of responses can enhance individual fitness by reducing the extent to which individuals compete with conspecifics for principal prey and, thereby, reduces the likelihood of cannibalism (frequent in coccinellids when prey are lacking [Dixon, 2000]). By performing rapid attacks individuals that grasp should also be better able to prevent responses by the ants attending *Caternaultiella* nymphs. Indeed, when attacked, these nymphs emit an alarm pheromone which also acts as an allomone attracting their attending ants, which in turn attack the *Anisolemnia* larvae (Dejean *et al.*, 2002).

In conclusion, the repertoire of behavioral patterns of *Anisolemnia* larvae, which includes a long sequence adapted to the capture of essential prey and a shorter sequence adequate for alternative prey, permits this predator to respond flexibly to novel situations represented by the encounter of alternative prey. This flexibility is supplemented by a kind of learning permitting these larvae to increase the possibility of success of their response.

ACKNOWLEDGMENTS

We are grateful to Dr. William R. Dolling (Brook Farm, Elstronwick, Hull, UK), and Drs. Barry Bolton and Robert D. Pope (The Natural History Museum, London, UK) for the identification of the Plataspidae, the Formicidae, and the Coccinellidae, respectively. We would also like to thank Andrea Dejean and Doyle McKey (University of Montpellier, France) for proofreading the manuscript. This work was supported by a grant from the French Ministry of Foreign Affairs (Project CORUS 2003-2006).

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