Use of Cobra Lily (*Darlingtonia californica*) & *Drosophila* for Investigating Predator-Prey Relationships

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In order to investigate predator-prey relationships in a laboratory, I have found the use of the cobra lily (*Darlingtonia californica*) and fruit flies (*Drosophila virilis*) an excellent experimental system. The experiments described deal with the feeding mechanism of a carnivorous plant and allow students to examine the impact of both predator and prey characteristics on the capture success of the predator. The approach described here can be used as a laboratory exercise or the subject for an independent study/research project.

Cobra lily is a carnivorous plant restricted in range to northern California and southern Oregon (Schnell 1976; Juniper et. al. 1989), but is readily available through biological supply houses (e.g. Carolina Biological Supply Co., 2700 York Rd., Burlington, NC). The cobra lily traps insects in a tubular, hooded leaf that curves in such a fashion as to resemble the head of a striking cobra (Figure 1). The leaves have small oval-shaped openings on the underside of the curved hood through which insects enter the trap. Extending below the opening is a forked appendage which is covered with nectaries, presumably for attracting prey. Insects can be observed to land on or crawl onto the "tongue-like" structure and then enter the trap. Once in the trap, escape is impeded by two mechanisms. In the upper, curved region of the leaf opposite the entrance hole, there are areas that lack chlorophyll and are known as areolae or fenestra. These areas are regions that permit light to enter the inside of the leaf, much like a window, and serve as light sources to confuse potential escaping prey by directing them away from the entrance hole. The other anti-escape mechanism is the downward pointing hairs which form a dense lining on the inner surface of the leaf tube. These hairs appear to impede insects from climbing up and out of the leaf tube. Under natural conditions, *D. californica* attracts a variety of prey items depending upon its location and the season. Prey items include: ants, grasshoppers, flies, moths and spiders (Juniper et. al. 1989).

Figure 1. Close-up of Cobra lily leaf showing tongue-like appendage and the opening through which insects enter trap. Note also the fenestra (see text) which appear as light specks on top of curved hood.

The cobra lily-fruit fly system is an excellent choice for investigating predatory-prey relationships in the laboratory for a number of reasons. The materials are relatively inexpensive and readily available to most schools. Both the insects and the plants require only minor care and are easy to grow and maintain in the laboratory. In addition, many students have an almost innate interest in exotic plants, and carnivorous plants in particular. The cobra lily with its unusual name and unique form is no exception. Other reasons for using this system are its versatility in investigating a number of different aspects of the predatory-prey relationship, the ease with which students can obtain sufficient data for meaningful statistical analysis, use of minimal laboratory equipment and its use in merging concepts of plant and animal biology.

Laboratory Procedure

Drosophila virilis is cultured in a fashion familiar to genetics students using prepared instant media and virtually any size bottles or containers. *Drosophila virilis* was used as the prey item in our experiments because they are somewhat larger than *D. melanogaster*, but there is no reason other insects could not be used if they are available.

Cobra lilies do not have extensive root systems and are readily grown in individual 9-inch plastic pots filled with a mixture of sand and peat moss. The soil mixture should be kept moist at all times. Additional plant care information can be found in Schnell

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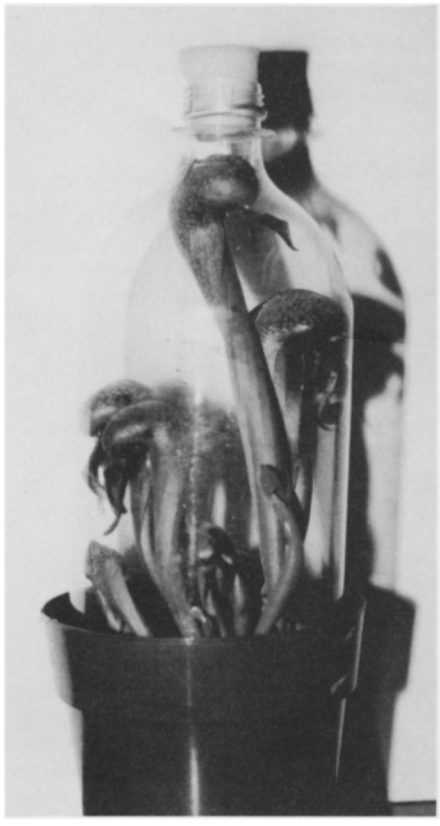

Figure 2. Experimental setup using 3-liter plastic container to enclose entire plant.

(1976). Plants at the College of New Rochelle are maintained in a greenhouse between experiments, but there is no reason a sunny windowsill would not work equally as well. During experimental trials when more controlled environmental conditions are desired, the plants are placed on a laboratory bench under fluorescent growth lights connected to a timer to provide a 12-hour photoperiod. Length of experimental trials varies, but most often a 24-hour period is used. After an experimental trial is complete, the plants are returned to the greenhouse for recuperation.

In order to provide background, stimulate interest and establish an intellectual framework for initiating experiments, assign a number of readings in the area of carnivorous plants and predator-prey systems. Heslop-Harrison (1978) provides a good introduction to the carnivorous habit in plants. Articles dealing with feeding behavior of the pitcher plant (*Sarracenia*) by Cresswell (1991), Wolfe (1981) and Plummer and Kethley (1964) will provide sufficient information to stimulate interest and help generate possible hypotheses for testing. Most general ecology textbooks (e.g. Smith 1990) and some introductory biology texts (e.g. Campbell 1990) provide sufficient background in the concepts of predator and prey systems, in particular, the role of prey density in predator success.

We use two different experimental arrangements, depending upon the experiment and the hypothesis under investigation. The first experimental setup employs a plastic 3-liter soda container with the bottom cut off to form a dome over the entire plant (Figure 2). The cut bottom of the bottle is pressed into the soil surface to prevent flies from escaping. The bottle cap is removed and replaced with a foam sponge or non-absorbent cotton plug. The plug prevents flies from escaping, allows ventilation during an experimental trial and provides a means for introducing an anesthetic to the flies at the end of a trial. Flies are etherized and counted; known densities of flies are introduced into the dome. Anesthetized flies are introduced into the dome on a piece of white paper placed on the substrate surface. After an appropriate trial length, usually 24 hours, the flies not trapped by the plants and thus still visible within the dome are counted, etherized and re-counted. Flies are etherized by placing large cotton swabs saturated with ether into the dome through the top opening. For those who prefer not to use ether because of obvious hazards, products such as Fly-Nap (Carolina Biological) should also work. By introducing different densities of flies into the dome, one can investigate the capture success as a function of prey density. The use of a 3-liter bottle to form a dome over the entire plant is simple and effective for examining the capture success of entire plants, but does not allow examination of individual leaves. In addition, students may encounter some difficulty in accounting for all the flies released within the dome.

An improved experimental design is shown in Figure 3. In this setup, individual leaves are enclosed within laboratory flasks. The size of the flask can vary, but 250-ml flasks appear to work best. The leaf is carefully inserted into the flask, the mouth of the flask is closed with a foam plug through which a hole has been bored, and the plug is cut to allow it to be slipped around the base of the leaf. Alternatively, non-absorbent cotton may be used to form a plug in the mouth of the flask and around the leaf base. The flask is held in place with a laboratory clamp and appropriate support stand. This experimental setup offers an advantage over use of the 3-liter bottles because uncaptured flies are readily visible through the wall of the flask, in many cases eliminating the necessity to etherize flies remaining in the flask at the end of an experimental trial. Accuracy of data collection is increased when the flasks are used because all flies not visible within the flask can be assumed to have been captured since there are no escape routes and no hiding places. The use of flasks also allows examination of capture rates of individual leaves. Therefore one may examine the role of

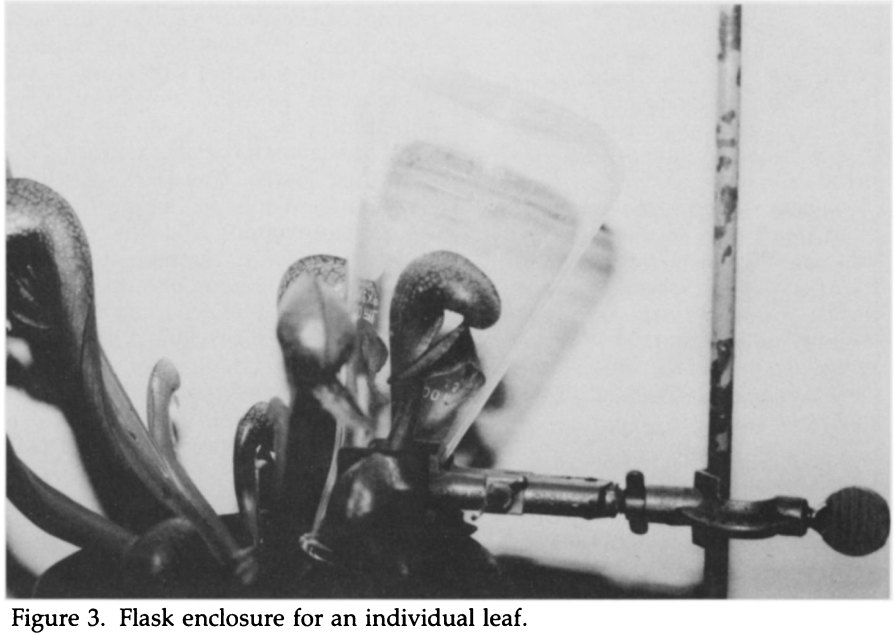

Figure 3. Flask enclosure for an individual leaf.

individual leaf characteristics (such as leaf age, size of opening, condition of tongue-like appendage) on the success of capture. Another important advantage to using flasks on individual leaves is that several replicates of the same experimental trial may be performed simultaneously on the same plant or, if plant material is in short supply, students may share plants and conduct their own experimental trials. Moreover, the use of enclosures on individual leaves allows students to view the capture rate as it happens, allowing time-course studies of capture.

Leaves of cobra lily are very successful at attracting, capturing and retaining flies. In our work, capture success of 100% for densities of up to 40 flies per flask for 24 hours is not uncommon. In fact, preliminary data suggest that the cobra lily operates in a strictly density-dependent manner as a so-called "type I" predator (Smith 1990), and in our work displays a mean capture success of 85.7% over densities ranging from 10–40 flies per leaf.

Suggested Experiments

A number of investigations are possible with this experimental system. The variety of hypotheses to be tested is limited only by the creativity of the investigators. I will provide a sample of some of the hypotheses we have investigated or plan to investigate.

The major thrust of most investigations using this system is to examine the factors that influence the success of the plant in capturing insects. Characteristics of the cobra lily that are likely to influence the efficiency of capture are:

1. Size of the leaf opening
2. Size of the leaf
3. Importance of the tongue-like appendage
4. Functioning of fenestra in preventing prey escape
5. The age of individual leaves.

Other factors that may also influence capture success are prey size, prey density and the presence of previously captured prey in the base of the leaf tube, as captured prey may provide olfactory clues to attract additional prey.

As a sample investigation, let us examine the influence of prey density on the capture success. In a laboratory setting, it may be best to have students work in groups in order to efficiently distribute the workload. To provide sufficient replication, a plant with a minimum of 4–5 leaves of similar size and general appearance is selected. The leaf height, head dimensions and diameter of opening for each leaf is recorded and care is taken to select leaves with similar characteristics. Individual leaves are carefully inserted into flasks, and flasks are held in place with clamps and a laboratory stand (Figure 3). Known numbers of anesthetized *Drosophila virilis* are placed into the laboratory flasks enclosing individual leaves. The flask is sealed with a foam sponge stopper (or non-absorbent cotton) and the plant is placed under a fluorescent growth light with a 12-hour photoperiod. At the end of a 24-hour period, the number of flies visible within the flask is recorded. The number of remaining flies is subtracted from the original number placed into the flask, and a percent capture success is calculated. The procedure is repeated for each prey density of interest. Other variables may be introduced into the experiment by removing the tongue-like appendage from leaves to test its importance in prey attraction, or covering the fenestra on the leaf hood to determine its importance in deterring prey escape.

Summary

The experimental setup described in this article allows for hands-on experience in investigating an important ecological concept, the relationship between a predator and its prey. It can be used to quantitatively analyze a variety of factors which may influence the ability of a predator to successfully capture prey. The techniques and materials employed are simple, inexpensive and readily adaptable to high school or college ecology laboratories.

Acknowledgment

The author would like to thank W. Mostafa for her work with the plants, R. Cassetta for thoughtful comments on the manuscript, and the College of New Rochelle for providing the opportunity to conduct the work. The manuscript also benefited from the careful and constructive comments of the reviewers.

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