Chapter 18

An Investigation of the Behavior of the Pea Aphid, *Acyrthosiphon pisum*

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Contents

Introduction

Aphids are versatile and fascinating laboratory organisms. They are easy to obtain, easy to rear, and are at once familiar to and yet poorly understood by students. The pea aphid, *Acyrthosiphon pisum*, is a large, pale-green aphid that lives on many plants of the pea family, including the long bean, *Vicia faba*. Like most aphids, the pea aphid displays cyclical parthenogenesis. In summer, all are female, producing live young by parthenogenesis from diploid, unfertilized eggs. The newly born nymph already has embryos developing inside her. She develops to adulthood in 2 weeks and gives birth almost immediately. This combination of the telescoping of generations and a very short life cycle leads to very rapid population increases under favorable conditions.

Summer females may be winged (alate) or wingless (apterous). Production of winged females — also by parthenogenesis — may be triggered by overcrowding or by deterioration of the host plant. Winged females can disperse to start a new clone on another host plant. In fall, changing photoperiod triggers the production of sexual males and females. After mating, the sexual female lays a few large, overwintering eggs. Each egg hatches into a female nymph who serves as a fundatrix, the first individual of a new clone.

Several aspects of the aphid life cycle may be used to illustrate or investigate interesting biological concepts. For example:

1. The rapid increase in population size on a new host from a single individual can be used to demonstrate the advantages of asexual reproduction.
2. Consideration of aphid life histories may lead to a discussion of the nature of individuality. Each aphid in a clone feeds, lives, and reproduces independently. However, members of the clone do not represent genetically distinct individuals and the whole clone in some sense represents a super-individual. These ideas are discussed in papers by Addicott (1979) and Janzen (1977).
3. Variations in plant quality, population density, and photoperiod can be used to investigate the environmental factors triggering the production of winged and sexual forms. Dixon (1973, 1977, 1985), who has written extensively on aphid biology and ecology, provides useful background information for students designing investigative experiments. Mousseau and Dingle (1991) discuss the factors triggering the production of winged forms.

The laboratory exercise described in this chapter investigates the escape behavior of the pea aphid. This investigation addresses the ultimate causation of behavior (its ecological function and adaptive significance) rather than proximate causation (the environmental or internal cues triggering the behavior).

To investigate the ultimate causation of behavior, it is necessary to assess what behavior is optimal (i.e., maximizing the animal's evolutionary fitness) under particular environmental conditions. This assessment requires a consideration of the costs and benefits associated with specific behaviors. The costs of a behavior may include the energetic costs of performing the behavior, the increased risk of predation that may result from performing the behavior, and the lost opportunity to perform other behaviors. A behavior benefits an animal by increasing its survival or reproductive success.

When an aphid is attacked by a predator, for example, a ladybug larva, she releases alarm pheromone from her cornicles. This pheromone signals predation risk to nearby aphids, who may be assumed to be genetically identical to the aphid releasing the pheromone. When an aphid is exposed to alarm pheromone, she has several behavioral choices:

1. Continue to feed, displaying no response to the pheromone.
2. Continue to feed but show agitation, kicking with her legs. This may prevent a small predator from carrying out a successful attack.
3. Stop feeding and walk from the feeding site.
4. Stop feeding and drop from the plant.

The costs and benefits of each of these behaviors can be assessed. An aphid which shows no response does not pay energetic costs or sacrifice feeding opportunity. However, she does not reduce her predation risk. An aphid showing agitation pays an energetic cost and may somewhat reduce her predation risk. An aphid walking from her feeding site pays an energetic cost for her actions and does not feed until she finds a new site and reinserts her stylet. She benefits from a reduction in predation risk. An aphid dropping from the host plant must locate and climb another host plant before she can resume feeding. She risks desiccation if she cannot locate a suitable host within a few minutes. However, she is no longer at risk from the original predator. Brodsky and Barlow (1986) and Clegg and Barlow (1982) have described the escape behavior of pea aphids in response to alarm pheromone. Dill et al. (1990) and Roitberg and Myers (1978, 1979) have assessed this behavior using a cost-benefit approach.

In the experiment described below, the costs associated with pea aphid escape behavior are altered by varying the quality of the host plant. Two groups of long bean plants are available. Half the plants have been watered regularly and represent good quality hosts. The other plants have been unwatered for at least 4 days. These plants represent poor quality hosts. Two of the behaviors — walking from the feeding site and dropping from the host plant — represent a loss of feeding opportunity and carry a lower cost to aphids on poor quality host plants. The benefits of reduced predation risk associated with these behaviors remain the same on good or on poor quality hosts. Thus, aphids on poor quality host plants would be expected to walk from the feeding site or drop from the host plant with higher frequency than aphids on good quality host plants. The actual experiment takes students approximately 30 minutes to perform.

Materials

Life Cycle of the Pea Aphid

Plastic petri dishes with moistened filter paper
Dissecting microscopes

Good vs. poor quality host plants:

Good quality host: one 3- to 4-week-old long bean plant
Poor quality host: one 6- to 8-week-old long bean plant
Maintain aphids on good or poor quality hosts for 3 weeks preceding the date of the exercise.

Crowded vs. uncrowded conditions:

Crowded conditions: Replace plants only once a week for the 3 weeks preceding the date of the exercise.
Uncrowded conditions: Replace plants 2 or 3 times a week for the 3 weeks preceding the date of the exercise.

Escape Behavior in the Pea Aphid (for 24 participants, working in pairs)

Long bean plants, 3- to 4-week-old (12 plants kept well-watered, 12 plants kept unwatered for 4–6 days before date of exercise; 24 plants total)
Magnifying glasses (12)
Fine forceps (12 pairs)
Paintbrushes, small (12)

Notes for the Instructor

The day before the exercise, 10–15 large pea aphids should be carefully placed on the top surface of one upper leaf on each long bean plant prepared for the exercise on escape behavior. The aphids can be selected from the host plants available for the exercise on the life cycle. Prod the aphids gently with a moistened paintbrush and wait until the aphid has withdrawn her stylet and started to walk from the feeding site before lifting her gently with the paintbrush to the experimental plant. The aphids will make their own way to the underside of the leaf and start to feed. It is necessary to transfer the aphids a day in advance of the exercise to give them time to assess the quality of their new host. Many students have considerable difficulty squeezing the aphid to obtain the pheromone-containing fluid. You may, if you wish, substitute a far easier but equally effective technique. The thorax of the aphid can be squeezed with a fine forceps to rupture the abdomen. Alarm pheromone is present in the exposed abdominal contents.

Emphasize two points to your students: (1) Wave the squeezed aphid over the experimental animals on the underside of the leaf immediately after pheromone release; the alarm pheromone is volatile and will dissipate within 10–20 seconds. (2) Wave the squeezed aphid directly over the experimental animals, within 1 or 2 mm, if possible.

The experiment can be modified by the addition of a control. Students could wave a forceps alone or a forceps holding an unsqueezed aphid over the experimental animals on both the well-watered and unwatered plants. Students could also be given the exercise as described and asked to design a suitable control themselves.

Student Outline

Aphids are small, soft-bodied insects that feed by sucking sap from the phloem of plants. As shown in Figure 18.1, the sharp, piercing mouthpart, or stylet, of the aphid can be inserted through the epidermis of the plant to tap the contents of a single sieve tube cell.

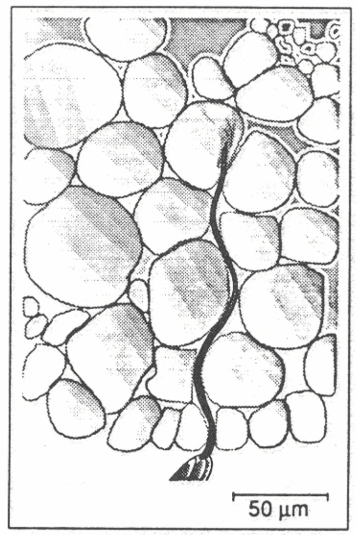

Figure 18.1. The inserted stylet of a feeding aphid.

Life Cycle of the Pea Aphid

Acyrthosiphon pisum, the pea aphid, is a large, pale-green aphid that lives on members of the pea family, which includes peas, beans, and clover. Like most aphids, *A. pisum* displays cyclical parthenogenesis. In summer, all aphids are female. They are viviparous, giving birth to live young produced from diploid unfertilized eggs.

At birth, the nymph already has embryos inside her. She starts feeding right away and reaches adulthood within 2 weeks. She can give birth immediately upon reaching adulthood.

Summer females may be winged (alate) or wingless (apterous). A female feeding on a young, uncrowded plant normally produces wingless females. Thus, a cluster of aphids seen on a single plant may represent clones of a single female.

The production of winged females, also by parthenogenesis, is triggered by overcrowding and/or poor quality food. The increased frequency of physical contact between aphids during overcrowding at a characteristic time in their development triggers the production of winged instead of wingless daughters. Poor quality food, from a mature or wilting plant, also stimulates the production of winged females. Winged females may escape from an overcrowded or deteriorating host plant and start a new clone on a fresh host plant.

In fall, the production of sexual males and females is triggered by environmental cues, principally photoperiod. Winged females are produced which disperse to new hosts where they give birth to wingless, sexual, egg-laying females. Winged males are also produced. Males disperse to find and mate with the sexual females which then lay a few cold-resistant, overwintering eggs. In spring, each egg hatches into a female nymph, who is the first individual of a new parthenogenetic line or clone.

1. Examine the bean plants on display in the lab. Note the relative abundance of winged and wingless aphids on good vs poor quality host plants and under overcrowded vs uncrowded conditions.

2. Remove a few winged and wingless females from a plant with a moistened paintbrush and examine them under a dissecting microscope.

Escape Behavior in the Pea Aphid

Aphids use chemicals called pheromones to communicate with other individuals of the same species. Pheromones are small, volatile molecules that are active in minute amounts. Insect pheromones may function as male attractants, territorial markers, or alarm substances.

We will examine the behavioral response of the pea aphid, *Acyrthosiphon pisum*, to alarm pheromone. When a pea aphid is attacked by a predator, she may produce an alarm pheromone to warn nearby aphids, usually members of the same clone. This pheromone is contained in fluid released through the aphid's cornicles (Figure 18.2).

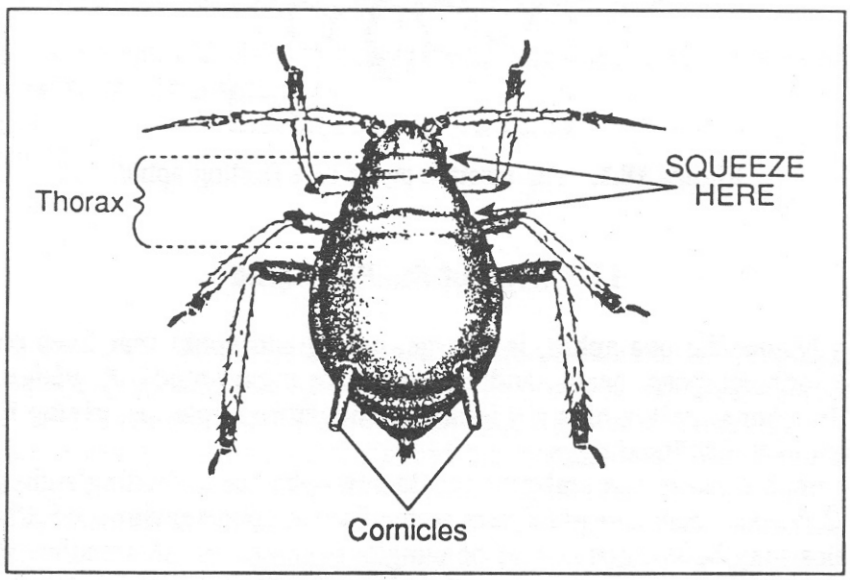

Figure 18.2. The pea aphid, *Acyrthosiphon pisum*.

When a feeding aphid detects alarm pheromone produced by a nearby aphid, she may react in one of four ways:

1. Show no response.
2. Appear agitated, raising and lowering her legs and vigorously waving her antennae, but continue feeding, not withdrawing her stylet from the plant.
3. Remove her stylet from the plant and walk away from her feeding position.
4. Remove her stylet from the plant and drop from the plant to the ground below.

Pairs of young bean plants have been set up in the lab. In each pair of plants, one is well-watered and the other has not been watered for at least 4 days. Each plant has had a number of pea aphids placed on one or two of its leaves.

1. Work in pairs for this exercise. Locate a leaf that contains a number of aphids on the well-watered plant. *Do not jar the plants or brush the leaves.*
2. Select a large aphid from the aphid cage on the bench. Squeeze the thorax of the aphid with a fine forceps, in the region indicated in Figure 18.2. If you are successful, a clear drop of fluid should exude from the cornicles of your aphid. Check your aphid with a magnifying glass and ask for advice if you are not sure that you have performed the operation correctly.
3. As soon as you are sure that your aphid has released pheromone-containing fluid, wave your aphid directly over the aphids on the underside of your chosen leaf for a period of 30 seconds. Two important points to note:
   (a) Wave the squeezed aphid under the experimental animals immediately after pheromone release. The alarm pheromone is volatile and will dissipate within 10–20 seconds.
   (b) Wave the squeezed aphid directly over the experimental animals, within 1 or 2 mm, if possible.
4. Observe the response of each aphid to the pheromone and tabulate the results in Table 18.1.
5. Carry out the same procedure with a fresh aphid if your well-watered plant has aphids on a second leaf.
6. Carry out the steps above for aphids on the unwatered plant, using fresh squeezed aphids. Again, tabulate the results in Table 18.1.

Table 18.1. Aphid response to alarm pheromone.

Aphid response	Well-watered plant	Unwatered plant
No response		
Agitation		
Walk		
Drop		
Total number of aphids		

Thought Questions

1. What are the costs and benefits to the aphid of (a) walking away from her feeding site? (b) dropping from her feeding site?

2. How might the costs and benefits of these behaviors differ for an aphid on a well-watered plant and another on an unwatered plant?

3. Pea aphids can respond in a number of ways to detection of alarm pheromone produced by a nearby aphid. In experiments carried out at Simon Fraser University, pea aphids were placed on well-watered plants under two experimental climatic regimes: hot and dry, and cool and moist.

Aphids were exposed to alarm pheromone and their behavior observed. Explain how the costs and benefits of dropping from the plant might be different for aphids under these two experimental climatic regimes. Would you expect aphids to drop more frequently under one of these regimes?

Techniques for Collecting and Rearing Aphids

General Information

The pea aphid, *Acyrthosiphon pisum*, exploits various types of host plants during the summer months, including clover and alfalfa. Aphids can be successfully transferred to long bean plants, *Vicia faba*, for rearing in the lab. Apterous (wingless) female aphids may be collected throughout the summer from natural or cultivated alfalfa or clover fields. Once clones become established in the laboratory, minimal care is required. Fresh plants should be provided every 2 or 3 days. Pea aphids kept on a long-day schedule (as detailed below) may be maintained as parthenogenetic clones indefinitely. Some clones at Simon Fraser University have existed in the asexual phase for more than 10 years. Each clone may consist of as many individuals as space and food will allow, providing an ample supply of specimens for laboratory exercises all year.

Host Plant

A continual supply of young bean plants (*Vicia faba*) is necessary to rear large numbers of *A. pisum*. Seeds and planting instructions may be obtained from any greenhouse supplier. Bean plants are easy to grow, requiring only light and regular watering. When raised in a greenhouse, plants should be ready for use as aphid hosts (at the 3-leaf stage) approximately 2 weeks after planting. Pea aphids may also be raised on “Alaska”-variety pea plants (*Pisum sativum*) with equal success (Harrison and Barlow, 1972).

Collection Site

Pea aphids are widely distributed across North America and are highly successful on various types of host plants (clover, alfalfa, pea, bean). Any wild clover or alfalfa field is a good potential collection site. However, local agricultural or pest management agencies should be contacted to determine whether *A. pisum* is found locally. If so, such agencies may be able to suggest collection areas or even provide aphids with which you may start your colony. Alternatively, cultivated clover or alfalfa fields (i.e., those used to produce livestock feed) are excellent sources of aphids, provided they are pesticide free. For further information on the distribution and identification of aphids, see Blackman and Eastop (1984) and Eastop and Lambers (1976).

Collection

Materials: sweep net, fine paint brush, collecting vials or petri plates with moistened filter paper, plant shoots, and wash bottle.

Technique

Apterous adults can generally be collected throughout the summer. However, highest numbers of aphids occur following at least 2 weeks of warm, dry weather. Heavy rain will knock aphids from their host plants and drown many of them, whereas clone numbers will be high after a period of dry weather.

Standard sweep netting technique can be used to collect these insects but may result in a large number of the individuals being crushed. Alternatively, the net can be held beneath a plant that is being shaken vigorously. Use a moistened paintbrush to gently transfer the aphids from the net to young plant shoots in individual vials or petri plates. This paintbrush technique is very effective since the aphids will stick to the brush and should begin feeding on the new leaf almost immediately after being placed there. Since aphids are fairly delicate and will die if their stylets are damaged, care should be taken when they are moved manually. To remove aphids directly from a leaf, gently prod them with a paintbrush. This will result in the retraction of the stylets and allow the aphids to be manipulated without damage. Ensure that the aphids are kept cool and have access to suitable leaves during transportation back to the laboratory.

Parasitoids

Many aphids found in the field carry wasp parasitoids which will result in the death of the adult aphid (Dixon, 1973). Wasp parasitoids oviposit in aphid nymphs, resulting in death of the latter within 7–9 days. Parasitized aphids swell, become light brown in color and adhere to the sides of the holding container or the underside of leaves, a process called mummification. Such mummies should be removed and destroyed immediately or held in isolation since the wasps that will emerge from them may destroy the clone. To facilitate removal of affected individuals, aphids should be held in separate containers (with fresh leaves) for at least 7 days following collection. During this time, the containers should be checked every 48 hours for the appearance of mummies. Surviving females may then be transferred to larger rearing cages.

Various aspects of the aphid-wasp interaction may serve as an interesting basis for other laboratory exercises or individual study projects for students. Wasps exhibit host discrimination, probing aphids and choosing to oviposit in those which have not previously been parasitized (Bai and Mackauer, 1990). Aphids have various defense behaviors which may protect them from wasp parasitoids (Gerling et al., 1990) and parasitized aphids may behave differently from unparasitized aphids when exposed to alarm pheromone (McAllister et al., 1990).

Rearing

Materials: rearing cages, young *Vicia faba* or *Pisum sativum* plants.

Any type of enclosure that is well ventilated and which allows light to penetrate is suitable for rearing aphids. When at low densities and on good quality plants, these insects will not migrate away from their host plants. However, higher density rearing or production of alates (winged females) requires the construction of individual cages, able to hold three or four plants at a time. Cages constructed of plexiglass, well ventilated with air holes covered in gauze or fine mesh, are preferred. These allow light to penetrate fully, resulting in healthier plants and more robust clones. These cages should be designed in such a way that technicians may easily replace the plants as needed. Alternatively, fine-mesh and wire can be used to construct an enclosure that can be placed around the host plants. Mackauer and Bisdee (1965) describe two such rearing devices.

Technique

Healthy (unparasitized) females should be transferred individually to young plants within the rearing cages (using a paintbrush as described above). Since adult aphids may produce up to 14 nymphs in a 24-hour period, clone size will increase rapidly. Plants which are dying or becoming too large for the cage should be replaced. Initially, when the clone is still small, all aphids should be removed from the old plant and transferred to the new one before the old one is discarded. However, once the clone becomes large, the old plant can simply be shaken over the new one (inside the enclosure), thus transferring enough aphids to establish a population on the new plant. Usually, after the colony is established, it will require new plants two to three times a week. To prevent the formation of alates, it is important to ensure that the plants used for rearing are young and healthy and that clone density is not excessively high. This will also help to prevent population crashes. In addition, to prevent the formation of sexual forms, aphids should be held under a 16-hour light, 8-hour dark cycle in the lab. A relative humidity of 60–70% is also recommended. Room temperature is acceptable for rearing. However, development time is temperature dependent (20°C days and 14°C nights are preferred) and extreme temperatures may result in a decrease in population growth rates (Harrison and Barlow, 1972). A fluorescent light source on a timer will provide adequate light to prevent the formation of sexual forms.

If alate production is desired, permit the aphids to become crowded by replacing plants only once or twice a week for approximately 3 weeks preceding the date at which the winged forms are needed. The clones should be checked regularly during this period because overcrowding may result in population crashes.

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Dr. L. M. Dill and Dr. B. D. Roitberg made the initial suggestions leading to the development of this laboratory exercise.

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APPENDIX A
Sample Data

Table 18.2. Aphid response to alarm pheromone
(data from General Biology 102, Simon Fraser University, Spring, 1993).

Aphid response	Well-watered plant	Unwatered plant
No response	414 (66%)	430 (54%)
Agitation	103 (16%)	130 (16%)
Walk	67 (11%)	127 (16%)
Drop	45 (7%)	102 (13%)
Total number of aphids	629	789

APPENDIX B
Chi-square Analysis of Experimental Data

A chi-square analysis can be performed on the data obtained in this experiment. Aphid responses to the pheromone can be combined into two groups. Aphids showing no response or agitation may be combined, since neither behavior results in a loss of feeding opportunity. Aphids walking from their feeding site or dropping from the plant do not feed for a period of time and may be combined to form a group that sacrifices a feeding opportunity.

The null hypothesis is that aphids on poor quality hosts will not sacrifice feeding opportunity (i.e., walk or drop) in response to alarm pheromone at a greater frequency than aphids on good quality hosts. Expected frequencies can be obtained by pooling data for all aphids.