
# The Adult Dipteran Crop: A Unique and Overlooked Organ

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## Abstract

The diverticulated crop is a unique and overlooked foregut organ in the Diptera that affects many physiological and behavioral functions. Historically, the crop was viewed simply as a reservoir for excess nutrients. The crop lobes and crop duct form an elaborate sphincter and pump system that moves stored nutrients to the crop lobes, oral cavity, and the midgut. The storage capacity of the crop lobes is significant when filled maximally and supplies sufficient carbohydrates to sustain prolonged activity and flight, and adequate protein and lipids to facilitate reproductive events. Crop emptying is under complex neuroendocrine and neural control and may be influenced by multiple neuromessengers, such as serotonin and dromyosuppressin. The crop lobes also serve as a site for the initial mixing of enzymes from the salivary glands and antimicrobials from the labellar glands with ingested food. These food-processing functions are associated with behaviors unique to dipterans, such as regurgitation (or bubbling), nuptial gift giving, and substrate droplet deposition or trap-lining.

## INTRODUCTION

In this review we survey the role of the crop organ in regulating hemolymph sugar levels, providing new insights concerning insect supercontractile muscle control, serving as an important reservoir for nutrients, as well as defensins, and serving as a reservoir for carbohydrates in both blood- and nonblood-feeding adults (**Figure 1*a***). We also review how the crop organ has become specialized in some dipteran groups for droplet formation, lekking behavior, and gift giving. Because the crop has not been well preserved in the fossil record, its importance to the evolution of the Diptera has been largely ignored; yet, the crop was as essential as the labellum for the episodic radiations that occurred, especially during the evolution of the angiosperms.

**Defensins:** small cationic proteins that function as defensive peptides against various types of pathogens

**Lekking:** a type of mating behavior in which groups of males gather and in which there is usually some sort of stimulus (e.g., pheromone) also attracts conspecific females for mating

**Diverticulated crop:** a crop formed as an offshoot or branch from the other parts of the digestive tract

## HISTORICAL AND EVOLUTIONARY LOOK AT THE CROP

### Phylogenetic Analysis of Crop and Evolutionary Importance

The ultimate significance of the diverticulated crop for the evolution of Diptera has been given insufficient attention (47). The role of the crop as a reservoir for nutrients in Diptera has been a major focus of research, possibly resulting in the neglect of its many other diverse functions. One of the first studies to report a diverticulated crop was by Dufour (28). An early reference (58) reported that most dipterans have a crop, but in some groups (Asilidae, Oestridae, and *Hippobosca* and *Melophagus* in Hippoboscidae) the crop is lacking. Of these three families, King (62) investigated only the Asilidae and reported the presence of a crop. The remaining two families are discussed below. King listed two differentiating characters that separate the dipteran digestive system from the mecopteran (and probably other insect orders) digestive system: a nonspecialized proventriculus and a diverticulated crop. The most common major synapomorphic trait listed in the literature for the Diptera is the presence of halteres, but as mentioned by Downes & Dahlem (24), the diverticulated crop was equally important for the order's expansion into diverse trophic niches.

The nature of the dipteran diet prior to the evolution of angiosperms is a topic of speculation. Downes (23) considered that the dual blood- and sugar-feeding behavior of some hematophagous flies relies on the possession of a crop, and this reliance may have predated the presence of early angiosperm flora. The classic and often quoted paper by Downes & Dahlem (24) established a trophic link between hemipterans (i.e., homopterans) and dipterans and hypothesized that, prior to the presence of angiosperms, flies obtained their main source of carbohydrates from honeydew. Although this may be true, Ráthay (89) reported that of the 135 insects visiting and feeding on the carbohydrates of the pycnidial nectar of rusts, 47% were flies. Consequently, at least two sources of carbohydrates were available to flies prior to the presence of the angiosperms. Under this scenario, responding to either liquid or dried carbohydrates using their tarsal chemoreceptors, flies would extend their proboscises and salivate and/or regurgitate liquid from their crop. Once the dried honeydew was dissolved, it was taken up and stored in the crop for later use. Thus, prior to the presence of angiosperms, both the proboscis (i.e., the labellum) and the crop were important for flies to obtain and store carbohydrates.

Once angiosperms evolved, brachyceran flies underwent a burst of rapid radiation (126). Support for flower feeding by the Brachycera during this period is provided by the fossil record on the basis of mouthpart morphology (90). Unfortunately, however, the crop and its contents do not preserve well, but it is without question that the crop played an equally important part in this episodic radiation associated with the appearance of flowering plants.

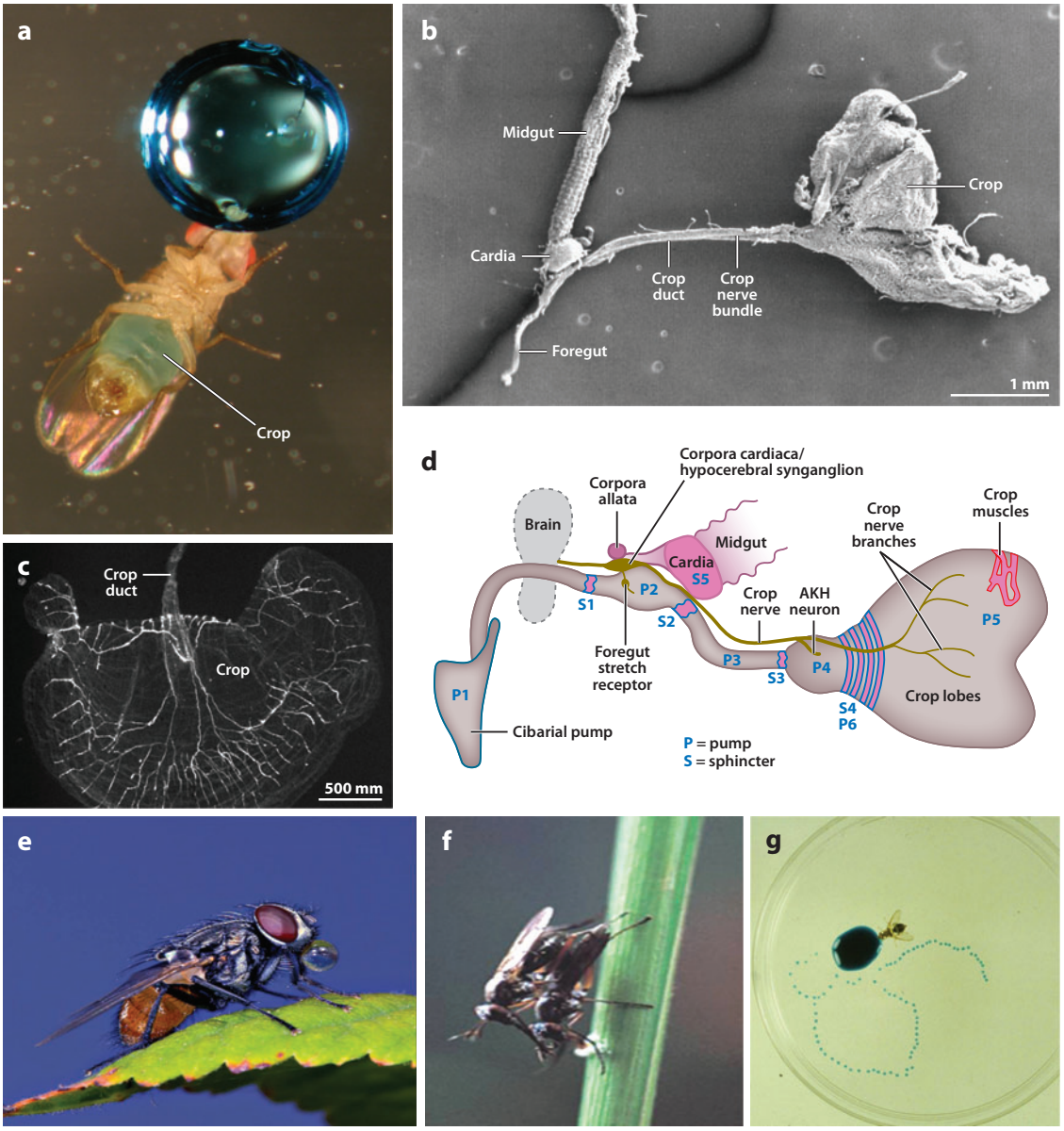


**Figure 1**

The dipteran crop. (*a*) An adult *Drosophila melanogaster* feeding on a blue-dyed sucrose solution; its nearly full crop is visible through the abdominal venter. (*b*) Scanning electron micrograph of the crop organ of *Phormia regina*. The foregut includes the esophagus and diverticulated crop system. The crop organ is composed of the crop duct, the crop lobes, or sac, and the crop nerve bundle. Posterior to the foregut is the proventriculus, or cardia, leading into the midgut. (*c*) Confocal microscopy image of the nerve plexus of adult *Musca domestica* coming from the expansion of the crop nerves on the crop duct onto the lobes of the crop, which is stained with a fluorescent antibody to dromyosuppressin. (*d*) Schematic modified from Reference 113 showing various sphincters, pumps, crop nerve bundle, and a small segment of the muscles of the crop lobes. (*e*) An adult *M. domestica* engaging in bubbling behavior. (*f*) Male of *Sepedon* sp., possibly *S. aenescens*, in Taiwan holding onto the antennal aristae of the female while the female feeds on the nuptial gift (photographed by S.-Y. Lee, but taken from **http://taipei.tzuchi.org.tw/tzquart/2006su/qs2.htm**). (*g*) Regurgitation behavior by *Bactrocera tryoni* forming from the crop a long trap-line of droplets of sucrose solution it has just ingested (courtesy of Alan Robinson).

# STRUCTURE

## General Anatomical Organization of the Crop Organ

**Crop nerve bundle:** a cable-like structure containing several axons that are housed in a single tube-like bundle and possibly contain different neuromessengers that have different effects on their target tissue or organ

**Synganglion:** a structure composed of several ganglia

**Antagomir:** a small piece of synthetically produced RNA used to silence endogenous microRNA

Considering that almost all fly species possess a crop, it is surprising to see that crop anatomy and morphology have been examined in so few species [i.e., *Phormia regina* (109), *Calliphora erythrocephala* (102), *Glossina brevipalpis* (80), *Drosophila auraria* (19) and *Bactrocera dorsalis* (71)]. The most definitive early study of the crop was by Graham-Smith (45), who mentions that a nerve plexus and tracheae supply the lobes of the crop. Other species for which innervated crops have been described include *Aedes aegypti* (14), *Glossina morsitans* (66), *G. brevipalpis* (80), *Musca domestica* (53), and *P. regina* (109). The origin of axons within the crop nerve bundle, at least in part, is presumed to be the corpora cardiaca (CC) (69).

The adult crop is diverticulated and consists of a bilobed sac (i.e., lobes) found in the abdomen and a narrow duct found in the thorax (**Figure 1*b***). In front of the cardia, the duct extends ventrally below the midgut but above the ventral nerve cord and associated thoracic-abdominal synganglion. The crop organ consist of four main structures: (*a*) epithelial cells producing the cuticular lining of the crop system, (*b*) the cuticular intima itself, (*c*) a pair of crop nerve bundles emanating from the CC and branching over the surface of the crop lobes (**Figure 1*c***), and (*d*) the crop muscles of the duct and lobes. These are anastomosed, supercontractile muscles connected by intercellular bridges (109) that form the various pumps and sphincters (113). Scanning electron (SEM) and transmission electron microscopy (TEM) of the muscles show tracheae, mitochondria, sarcoplasmic reticula, and glycogen.

## Exceptional Crop Anatomies and Anomalies

The crop lobes are reported to function in capacities other than storage of nutrients in some phorid flies (20) and in one species of fruit fly (78). The crop lobes are reported to function in capacities other than storage of nutrients in some phorid flies (e.g., function not reported but called Dufour's glands) (20) and in one species of fruit fly (contains a lekking pheromone) (78). In addition to these two reports, a few references in the literature suggest anomalies in the substances found within the crop or unusual dispatching of nutrients. Sang et al. (95) reported that tsetse flies (*Glossina morsitans centralis* and *G. m. morsitans*) infected with the salivary gland hypertrophy virus showed blood clots in the crop lobes, which is not normal. Bryant et al. (11) demonstrated that microRNA miR-275 was essential for normal blood digestion and, by injecting *A. aegypti* with its specific antagomir, the blood meal was pushed from the midgut, its normal destination site, into the crop lobes. They attributed this to a faulty sphincter between the foregut and hindgut. Disney (20) reported hairs and scales, presumably of arthropod origin, in the crop of female *Megaselia rufipes*. Moloo & Kutuza (80) found blood clots and crystals in the crops of tsetse (*G. brevipalpis*) fed in the laboratory. They also reported that crop lobes were distended with air displaced from the midgut and crop lobes after blood feeding.

King (62) questioned the presence of a crop in the Phoridae. Disney (20), however, wrote they possessed a crop and reported an interesting situation concerning crop morphology. In his paper, Disney reported that Dufour (28) described an unusual mechanism associated only with the full crop of female phorids in which the tubules associated with the crop uncoil and are immersed in the fluid contents of the crop. No further studies of this unusual crop structure have been reported for the phorids.

## REGULATION OF CROP FILLING/CROP EMPTYING AND INVOLVEMENT IN SHORT-TERM/LONG-TERM FEEDING

The crop organ is a mechanical, muscular system of pumps (P) and sphincters (S) (**Figure 1*d***) independent of direct nervous control (37, 63, 109) operating on the principles of hydrodynamics (114). Flow of materials within the crop is driven by two pumps located at either end of the system. The anteriorly located cibarial pump brings fluids into the foregut/crop/midgut, and the abdominally located crop lobes pump fluids forward or out of the system. The direction of flow within this system depends on where the hydrostatic pressure is the greatest (113).

In the well-characterized crop system of *P. regina*, muscles of the crop lobes and those at the posterior end of the crop duct function as the major pumps of the system (P5 and P4 in **Figure 1*d***). P4 is considered a major pump because muscle fiber diameter is greatest in this region of the crop duct. P4 is also responsible for pushing fluids either forward to P2 or backward to the crop lobes (P5). Between P4 and P5 is a series of circular muscles functioning both as pump (P6) and sphincter (S4). Other pumps and sphincters of the duct are involved in moving the diet forward or backward to the crop lobes (**Figure 1*d***). This complete system functions as a contained hydrostatic unit where the diet initially enters by the action of the cibarial pump. Fluids are moved forward and can enter the midgut via S5, which is the cardiac sphincter. During filling, the food is moved posteriorly toward the crop lobes, whose muscles were suggested to be activated by stretch-activated channels (115). A mechanism similar to that of stretch-activated muscles has also been proposed for the various sphincters and pumps. Although no experimental proof has substantiated the ideas put forth by Thomson & Holling (114), the performance of the pumps and sphincters is consistent with the observed magnitude and frequency of crop lobe contractions (113). As the crop lobes fill, their rate of contraction increases, reaches a peak volume, and then decreases (112). Establishing that the crop lobe muscles are activated by stretch-activated channels was accomplished by using a peptide toxin that specifically blocks them (109).

When the crop is fully distended, the pressure within the system is so great that S3 and S4 are always open and P4 is completely full. The rate of crop emptying is influenced by the metabolic rate of the fly, locomotor activities such as flight, and the osmolarity and composition of the hemolymph. In *P. regina*, the contraction rate reaches a peak at about 6 μl and then declines as the crop volume increases. If the crop volume reaches a sufficient amount (≥6 μl) (106, 112), the fly will regurgitate the crop contents, a behavior known as bubbling (**Figure 1*e***), but if the crop contains an insufficient amount (≤6 μl), most flies will not bubble (106).

The value of 6 μl, which is essential to elicit bubbling in *P. regina* (106), is the same as that shown to produce the highest rate of contractions of the crop lobes (112). Thus, the crop volume producing the greatest number of contractions for different fly species may be the value at which bubbling ensues. Even though crop fluid concentration may not initiate bubbling (68), bubbling behavior results in the loss of water from the diet via evaporation and ultimately produces a more concentrated crop content (56, 68). When the crop volume, expressed as P2-filling frequency, decreases (value not known), the fly resumes foraging and, if it contacts another food source, commences to fill the crop. To prevent negative or contrapressure from the crop lobe contractions of the previous meal from filling in a subsequent meal, *P. regina* has in place two compensatory mechanisms. First, a series of circular muscles acting as both pump (P6) and sphincter (S4), plus the involvement of P4, S3, and S4 (115), negates the crop lobe contraction effect or contrapressure on the filling process (113). Second, the neuropeptide dromyosuppressin (81), shuts down the crop contraction rate (53, 81, 91). Together, these two compensatory mechanisms prevent negative pressure from crop lobe contractions during any meal subsequent to the first meal and with food already in the crop.

**P:** pump

**S:** sphincter

**Stretch-activated channels:** ion channels activated by physical stretching of the plasma membrane

**Bubbling:** a behavioral process whereby the fly pumps liquids from the crop lobes onto the tip of the mouthparts and produces a droplet, not a bubble

**Bubble:** liquid in the form of a droplet pumped from the crop lobes

**AKH:** adipokinetic hormone

*Drosophila melanogaster* has three different myotropins, in addition to drosulfakinin 0, and each has a different effect on the crop system (60, 85). Unfortunately, differences in experimental techniques prevent direct comparisons between crop system function in *P. regina* and *D. melanogaster*. Experiments with *D. melanogaster* have focused only on contractions at the base of the crop duct, which in the *P. regina* system would be P4 and not the crop lobes. On the basis of the differences observed between these three myotropins, the investigators suggest both their synthesis and release may be under different sensory inputs. If this is true, it makes a strong case for separate neural control systems in the crop, especially for *Drosophila subobscura*, in which the male can control crop emptying and shares its crop nuptial gift with the female. Another possibility for their differential effects is that under certain circumstances, the crop can be shut down temporarily for shorter or longer periods, and this somehow facilitates some behavioral/physiological function(s) not associated with the foregut or digestive system.

The contraction rate of the various pumps relates directly to emptying of the crop (114). The contraction rate is responsible for fluctuations of pressure change within the system (114). Whether some of these other pumps are innervated, as we now know is true for the crop lobes in *D. melanogaster*, *P. regina*, and *M. domestica*, by neurons carrying the dromyosuppressin-like peptide remains to be demonstrated. P4 has not been investigated extensively, but studies show a neural branch going to that region. If *P. regina* fills its crop lobes to a volume beyond their greatest contraction rate, where the contraction rate caused by the stretch-activated channels is much greater than 6 μl for the fly, the ability of P4 to move fluids forward and out of the lobes is reduced or compromised. To compensate for this low contraction rate of P4 and P5, studies have shown that P4, which is involved in moving the fluid either forward toward the mouth/cardia or backward into the crop lobes, can be activated and contraction rates increase (75). It has been suggested, but not demonstrated, that activation of P4 also relies on stretch-activated channels (117). P4 is modulated by an exogenous source of serotonin in the hemolymph, which causes an increase in the rate of P4 and is believed to originate from the large serotonin neural plexus of the thoracic-abdominal synganglion (75). Thus, a full crop with P4 activated by serotonin will push the diet forward into P2—at this point S1 and S2 are involved—and P2 either pushes the diet into the midgut or out in the form of a droplet. The discovery of both insulin and adipokinetic hormone (AKH) pathways emanating from the CC and contacting P4 may be another way of regulating hemolymph solutes such as glucose and amino acid concentrations (109). Gelperin (37) showed that increasing the blood solute level of the hemolymph slowed down crop emptying, and Thomson (113) demonstrated a decrease in the rate of opening of S2 by increasing the solute concentration of the bathing media (i.e., more slugs of diet or more peristaltic waves from P3 were required to open S2). Thomson (113) also suggested that attention should be given to how the hemolymph characteristics affect S2.

Crop emptying is independent of cephalic nervous control in flies delivering both sugar and proteinaceous nutrients to the crop. When filled to repletion with either nutrient type, the crop of *P. regina* emptied over a similar time course and with a similar shaped curve (107). The rate of emptying is dependent on the volume consumed and on the concentration of the nutrient imbibed. This suggests the mechanism(s) for crop emptying in nonblood-feeding flies is somewhat similar for both nutrients. Studies agree that the regulation of the pumps and sphincters causes the crop to empty (80, 113, 116). We know little about the mechanisms regulating sphincter control in the fly foregut, but one study suggests a chemosensory, phagostimulatory mechanism is responsible for regulating the crop sphincter in *Culiseta inornata*; however, the mechanism(s) of control in other dipterans needs to be investigated (98).

The physiological bathing medium or hemolymph can also affect crop emptying. Increasing the osmotic pressure of the hemolymph (37, 99, 122), temperature (80), intactness of the crop

(63, 80), and growth of the peritrophic membrane (51) all affect crop emptying in several dipteran species. One question is whether the addition of the hemolymph sugars, glucose and trehalose, has any effect on the rate of crop lobe contraction. A series of experiments that separated osmotic from chemical recognition effects demonstrated the effect was chemical (i.e., glucose decreased the rate of crop lobe contraction whereas trehalose increased it) (112). This recognition effect is on the muscles directly because the experiments (112) were done *in vitro* on completely isolated crops. Thus, the rate of crop emptying is influenced by the volume of the crop and blood solute concentrations (37) and is independent of direct nervous control (37), but it is modulated by some peptides (109) and at least one biogenic amine (75).

**CC/hypocerebral complex:** a single, complex structure in the Diptera formed by the fusion of the corpora cardiaca and the hypocerebral ganglion

**Median abdominal nerve (MAN):** a part of the ventral nerve-net of *Phormia regina*

An additional aspect of crop filling/crop emptying is crop involvement in both the short-term and long-term cessation of a meal (16, 37, 38, 40, 41, 100). Cessation of short-term feeding (i.e., a single meal) in flies is due to distension of the abdomen, caused by either a large blood meal (48) or a full crop (17). Stretch receptors monitor abdominal distension and provide negative feedback to the central nervous system, which effects the central excitatory state and peripheral receptor thresholds to stop feeding. For the abdominal feedback, the ventral nerve cord is transected at different places to elucidate which ganglia are most important. Once severed, hyperphagia, or overeating, results; if the crop is filled beyond maximum capacity, it can burst. Removing input from the body-wall receptors produces greater hyperphagia than does removing negative feedback from foregut receptors on P2 (17). Using the same type of nerve transection, Gwadz recorded similar results for blood feeding in mosquitoes: hyperphagia and midgut bursting (48).

Expansion of the crop lobe volume causes stretching of the abdomen and the ventral nerve-net in *P. regina*. Located at the junctions of the median abdominal nerve-net are nonadapting stretch receptors, which while stretched continuously send negative-feedback information to the thoracic-abdominal synganglion, resulting in meal termination (39). The arrangement of nerves involved in sensing stretch of the abdomen probably differs among fly species, and caution should be exercised when using the *P. regina* model to fit other species. Once the crop fills to a critical volume and the meal is finished, various behaviors take place depending on the fullness of the crop. If the crop contains a large volume, the fly may bubble to reduce the water load or engage in other types of behaviors. Regardless of crop volume, slugs of fluid are pumped forward and cause P2 to increase in volume. On each side of P2 are two foregut stretch receptors that are anchored at their terminal ends to the wall of P2 and are connected at their proximal ends to the CC/hypocerebral complex, which is in turn connected to the recurrent nerve. As P2 fills and increases in volume, the two foregut stretch receptors are stretched and activated (41) (**Figure 1*d***). This constant filling and emptying affects the stretching of the two receptors on each side of P2 and is one way for the fly to monitor how much fluid remains in the crop. Thus, it also alerts the fly to how fast the crop is emptying. A crop can be full of nutrients, but if the concentration of sugar is high, it will empty slowly. Thus, feedback from this area affects long-term feeding regulation. Transection of the recurrent nerve going to the brain results in sugar hyperphagia (17) and protein hyperphagia (6), and one of its effects is alteration of the tarsal acceptance threshold (increasing the fly's unwillingness to feed on solutions of lower sugar concentration) (29). Similar transections of the median abdominal nerve (MAN) in *P. regina* failed to alter or influence tarsal acceptance threshold, even though hyperphagia results; thus, its influence is central and not peripheral (29). Edgecomb et al. (29) reported that MAN stretch receptors monitor crop volume and crop muscle contractions, but found no evidence that crop muscle contractions are affected. Consequently, we know of no study confirming that MAN monitors crop muscle contractions, but evidence does support volume effects. *P. regina*, like other nonblood-feeding flies, must also be able to monitor crop contents composed of proteinaceous nutrients. It is hypothesized, as with sugar ingestion,

protein ingestion into the crop is also regulated by abdominal stretch, which in turn is influenced by the crop volume (7).

**Trophallaxis:** the direct mouth-to-mouth transfer of fluids from the crop of the male to the crop of the female

The literature shows that crop emptying can result in nutrients being pushed out of the crop lobes and into the midgut for digestion, moved forward out of the oral opening and retained on the mouthpart (bubbling regurgitation) (56, 106), moved forward out of the oral cavity and dropped as a trap-line (13) or lekking pheromone (78), or shared with a female via trophallaxis (103), all of which are a form of regurgitation of crop contents. Considerable effort has been given to understanding the different inputs or inhibitions acting on the crop system, yet we know little about the neural circuitry or signaling pathways involved.

## PHYSIOLOGICAL FUNCTIONS OF THE CROP

### Eclosion Involvement of Air in the Crop

Hematophagous (70) and newly emerged (122) flies often contain air bubbles of different sizes in the crop. The reasons and function(s) for air storage in the crop are not well understood. Normally, the crop lobes in posteclosed adults are located entirely within the abdomen. Two studies, however, reported cases in which the crop in *Stomoxys calcitrans* appeared reduced and was located in the thorax rather than the abdomen (70). This is interesting because Marshall & Staley (79) reported that crop lobes in newly emerged mosquitoes were initially located in the thorax and a large air bubble in the midgut was slowly pushed forward into the foregut and then into the crop, where the bubble was involved in pushing the unexpanded crop from the thorax posteriorly into the abdomen and facilitating expansion. This entire process took 12 to 22 hours to complete, and they also reported that air remains in the crop for 48 hours after pupal emergence. Coinciding with the expulsion of air from the midgut into the crop of mosquitoes is hypopygial rotation of the male's genitalia by 180° (79). To date, few studies have examined the mechanism(s) controlling hypopygial rotation in dipterans. O'Donnell & Klowden (84) demonstrated that removing neural input had no effect on hypopygial rotation, but that methoprene administered to fourth-instar larvae and early-instar pupae slowed rotation. Marshall & Staley (79) intimated the mechanism might be the movement of air from the midgut into the crop.

In newly emerged and unfed tsetse flies, the midguts and later the crops are filled with air, which keeps the crop from collapsing. In most dipterans (31), the air sacs help maintain abdominal shape. Tsetse, unlike other flies, lacks abdominal air sacs (118). As the flies feed and blood enters the crop, the air in the crop diminishes, but where the air goes and how it is regained are not known (80). Lee & Davies (70) suggested that the air space observed by Moloo & Kutuza (80) might have been the air sacs rather than the crop, and their study on *S. calcitrans* showed no air in the crop. The study by Tobe & Davey (118), however, refutes the suggestion by Lee & Davies (70) and confirms that the air space observed by Moloo & Kutuza (80) in tsetse was in fact the crop.

### Regurgitation (i.e., Bubbling or Droplet Formation) and Vomiting

The terms regurgitation and vomiting are used loosely in the entomological literature and, if used incorrectly, may cause confusion when determining the mechanism(s) of voiding nutrients and pathogens. This also applies to the term mating trophallaxis, which in the Diptera is a type of regurgitation from the crop.

Regurgitation is the expulsion of food from the mouth, pharynx, esophagus, or crop. In both vomiting (i.e., when food materials are pushed out of the midgut into the foregut and then are expelled from the oral opening) and regurgitation, the material expelled must pass one or more

sphincters before leaving the opening of the mouth. Reports on malfunction of a sphincter leading to vomiting have been documented only in hematophagous insects (mosquitoes and sand flies) (11, 124). Graham-Smith (44) noted, "When feeding on sugar, small areas are moistened either with saliva or, in the case of flies fed on fluids, with vomit." If flies already have food in the crop and they feed, especially on dry foods, they will regurgitate, not vomit, the crop contents. A new classification of feeding that directly involves the crop has been proposed for *Bactrocera* species. Fluid-centered feeding involves species having a short hypopharynx; thus, saliva is not delivered directly onto the food but is added to the food while it is taken up into the crop. Flies feeding in this manner also have fine micropores of the pseudotrachea and prestomal spines (not teeth), which are used for filtering the ingested fluid, thus avoiding ingestion of large particles. It is proposed that flies feeding in this manner use the reverse flush of fluids or regurgitant from the crop to clear the filtering structures of any large particles not imbibed with the diet (123). Lowne (77) reported that flies usually regurgitate their food prior to it entering the midgut; thus, the regulatory mechanisms for regurgitation and vomiting are different. Having fluids in the crop is essential for regurgitation and bubbling (77, 106).

Straif et al. (110) stated that for *S. calcitrans* "transmission by regurgitation has been neglected for a long time." All the reports on *S. calcitrans* indicated vomiting. Regardless, we know almost nothing about the mechanisms and/or stimuli leading to either regurgitation or vomiting in adult flies. The crop is surely involved in regurgitation (106), but its involvement in vomiting remains unknown. Using TEM, Volf et al. (124) reported that the stomodeal valve in sand flies and *Culex* mosquitoes transmitting avian trypanosomes was compromised, and stated "the phenomenon involving a blocked valve facilitating the regurgitation of parasites into the vertebrate host may occur generally in heteroxenous trypanosomatids transmitted by the bite of nematoceran Diptera."

Nonblood-feeding flies eliminate excess water from meals mainly by bubbling (**Figure 1*e***) or regurgitation, and hematophagous flies generally eliminate water via rapid diuresis (9, 25). While bubbling, nonhematophagous flies eliminate water and the content of the crop becomes more concentrated (56, 107). The main reason for unloading the water from the meal is to increase flight efficiency. This has been demonstrated in *Glossina swynnertoni*, whose flight speed after a blood meal decreased from 15 to as low as 3 miles per hour (42). This inability to rapidly locomote or take flight has been demonstrated in mosquitoes (92).

## Reproduction-Related Storage of Protein Meal in the Crop of Nonhematophagous Flies

Adult, nonblood-feeders obtain their amino acids for adult reproductive development either from their larval stores (autogenous) or from an exogenous source of nutrients (anautogenous). These autogenous flies still frequently need, however, a carbohydrate meal for general metabolism/flight. Oftentimes, autogenous flies need a blood meal or proteinaceous meal after depositing their first batch of eggs. If blood is the main protein meal, it is generally stored in the expandable midgut and does not enter the crop. Nonblood-feeders generally have a nonexpandable midgut and also use the crop as the storage reservoir for proteinaceous diets (e.g., dung, animal exudates, decaying tissues, and rotting plant materials) (121). The reproductive significance of the crop can be appreciated when considering that one full meal of a protein-rich nutrient is enough for female *P. regina* to produce a normal complement of eggs (107). Thus, the crop for nonhematophagous flies is similar to the midgut of hematophagous flies with respect to its ability to store large quantities of nutrients destined for reproduction. We are not sure whether adults feeding as predators (e.g., asilids, empids, and some ceratopogonids) move the diet directly into the midgut or partition it by moving the overflow into the crop.

## Crop Storage of a Carbohydrate Meal in Adult Dipterans that Diapause

**Vessel-feeders:** blood-feeding flies that penetrate and feed on blood inside the capillary

**Pool-feeders:** blood-feeding insects that lacerate the capillaries and feed on the blood that emerges

The main carbohydrate meals of adult dipterans are pycnidial nectar (89), honeydew (24), extrafloral nectaries (55), flora nectar (47, 83, 105), and sometimes nonplant nutrients from wild yeast (4). In most of these cases, the carbohydrate meal is dispatched to the crop. Few studies have examined the importance of the crop as a storage site for carbohydrates in flies preparing for or in diapause. Diapausing *P. regina* females had crops completely full of a thick-syrup, sugary substance (106), and the crop stored these sugary substances in sugar-feeding mosquitoes diapausing in winter (23). By comparing the quantities of sugar consumed between two species of blow flies (i.e., diapausing and nondiapausing), Greenberg & Stoffolano (46) showed that diapausing species consumed significantly more sugar. Studies showed that female *Culex pipiens* did not bite in preparation for hibernation but instead produced fat body hypertrophy by feeding on sugar (8). In overwintering adults, most of the water from nectar is probably removed, at least in nonblood-feeders, by regurgitation and reingestion. How mosquitoes preparing for diapause remove water from crop nectar has not been reported.

## Switching Mechanisms, or Factors Involved in Diverting Nutrients in Hematophagous Dipterans

Numerous investigators examined the way in which hematophagous flies divert the diet solely to the midgut, the crop, or both. Arguments have been presented favoring a chemical detection mechanism (33) for nutrient switching in mosquitoes. Friend (35) recognized three behavioral modes of feeding in mosquitoes: blood feeding, nectar or sugar feeding, and water consuming. He reported that diet destination, which normally depends on the phagostimulants present, is influenced by these behavioral modes. In the blood-feeding mode, blood usually goes to the midgut when the mouthparts are piercing a substrate. In the sugar-feeding mode, sugar solutions go to the crop when the mouthparts are imbibing liquids that are not under a membrane. In the drinking mode, small amounts of water go to the midgut, again when the liquids are not under a membrane. For *Culiseta inornata*, a vessel-feeder (35), and *Tabanus nigrovittatus*, a pool-feeder (36), it was possible to switch the destination of the diet by presenting the diet using a feeding mode and diet composition different from what the fly would experience in nature. In these studies, the authors concluded that, in addition to feeding mode, temperature, phagostimulants present, and osmolarity of the diet can influence diet destination. Friend (35) concluded that a feed-forward mechanism, modulated primarily by phagostimulants present and feeding mode, determines meal destination.

Meal destination has been reviewed elsewhere (43, 119). The most comprehensive table showing diet destination in about 35 species of bloodsucking dipterans is available in Reference 70. It is generally accepted that if a fly has a distensible midgut (batch digestion), the blood meal and dilute sugar meals go to the midgut and the more concentrated sugar meals go the crop. In nonblood-feeding flies with a small and nondistensible midgut, the diet, regardless of makeup, fills the midgut if it is empty and then the diet goes to the crop. There are exceptions. *Glossina* sp., *Stomoxys* sp., and *Hippelates pallipes* put large amounts of blood into the crop (43). An often overlooked paper by Schmidt & Friend (98) showed that diet destination in *C. inornata* is controlled by a "differential, chemosensory-based control" of the crop and midgut sphincter, and suggested that receptor involvement is based on two different stereospecific sites responding to pyranose and furanose. They also noted that chemosensory control of ingestion is different from diet destination and that the rigid sucrose specificity of the crop ensures any energy-rich diet goes to the crop. This appears to be the first chemically controlled mechanism for valve/sphincter control suggested for

the dipteran crop. Day (14) suggested that nervous control by the buccal cavity receptors sent the response to the stomatogastric nervous system, which in turn controlled contraction of the sphincters involved in diet destination. Overall, the literature suggests that diet destination in the Diptera is controlled by combinations of physical factors (temperature and osmotic pressure of the diet), chemical phagostimulants, and nutritional factors (98).

## Glandular Contribution to Crop Contents

Most enzymes identified from crop contents are derived from the salivary glands and are involved in the initial stages of nutrient digestion. Sinha (101) concluded that four enzymes in the crop were not found in the salivary glands but were produced by the crop. This finding is questioned because the literature does not support the crop producing its own enzymes. Midgut enzymes are not found in the dipteran crop. The panorpoid ancestors, based on extant species, are believed to differ from holometabolous insects where digestive enzymes never passed forward from the midgut to the crop. Midgut enzymes in the panorpoid group, which includes the Diptera, are not found in the crop, and the panorpoid ancestors differed from those of the holometabolous ancestor in which digestive enzymes passed forward from the midgut to the crop (111). This appears to be true, and attempts to find trypsin-like proteases in the crop of flies have failed (86). Only in exceptional cases, in which the stomodeal sphincter appears compromised, is material vomited from the midgut and put into the crop (11, 124). Bryant et al. (11) reported that the blood in their study was pushed from the midgut into the crop, whereas Volf et al. (124) made no mention of blood being shunted into the crop in sand flies that have a defective cardia and/or stomodeal sphincter. A *drop-dead* mutant of *D. melanogaster* has a defective gut function, probably due to a malfunctioning stomodeal valve and to the presence of an enlarged crop having a higher than normal contraction rate (88).

Usually, salivary gland secretions, as well as nutrients ingested, end up in the crop and some digestion, especially of carbohydrates, occurs there (33, 49). Lester & Lloyd (74) noted that the powerful anticoagulant in tsetse is important because it prevents coagulation of the blood meal in the crop, and Rossignol & Lueders (93) reported that a salivary bacteriolytic factor ends up in the crop of mosquitoes. One must be cautious of claims about the source of digestive enzymes, because Dillon & El Kordy (18) reported that for *Phlebotomus langeroni* that seven α-glucosidases were found in the midgut and not in either the crop or the salivary glands. This finding differs from many studies reporting the salivary glands produce the carbohydrases, which then end up in the crop where sugar digestion begins. Recent studies show that the labellar glands of some flies are involved in the production of antimicrobials (e.g., antibacterial peptides) (32) and that their secretions also end up in the crop. The importance of the labellar glands has not been appreciated, and their regulation and deposition site(s) in flies are generally not known (21).

It was suggested that the diverticulated crop of mosquitoes is important because it keeps nectar, which might contain proteinases capable of inhibiting blood digestion, away from the midgut (42). Obligate blood-feeders such as tsetse presumably lack labellar glands and feed on closed vascular blood containing fewer pathogens, compared with blood-feeding or nonblood-feeding flies feeding on open sources of nectar or blood. Thus, any pathogens in the nectar or blood of non-vessel, blood-feeders are dealt with by many antimicrobial immune genes expressed and upregulated in the midgut (72) or cardia (50), whereas dipterans feeding on open carbohydrate/proteinaceous sources require labellar/salivary gland secretions in the crop to deal with pathogens.

Schlein & Warburg (96) stated that "free-solution sugar-meals which may contain contaminants, enter the crop where they mix with the antibacterial factor." Schlein (97) calls the sand fly crop a sterilization organ on the basis of their 1985 (96) study, in which they demonstrate an

antibacterial factor in the crop and note that when the sand fly is in the blood-feeding mode and inserting its proboscis into a tissue, whether animal or plant, the sand fly's meal contains few if any pathogenic microbes and the meal goes directly to the midgut. When the sand fly is surface feeding for carbohydrates, however, the meal goes to the crop, where Schlein suggests antimicrobials destroy any pathogenic organisms consumed.

**Demethylchlordimeform (DCDM):** an octopaminergic agent that causes hyperphagia when injected into *Phormia regina*

It is believed few, if any, pathogenic organisms of vessel-feeding, hematophagous flies (e.g., mosquitoes) are imbibed with the blood meal into the midgut, but that pathogens may enter the crop during carbohydrate feeding. This is not true for non-vessel, blood-feeders/pool-feeders such as tabanids. Lehane et al. (73) failed to find the two defensins, Smd1 and Smd2, isolated from the midgut in the crop of *S. calcitrans*, which is not surprising because the crop mainly stores bacteriolytic factors produced in the salivary glands and the midgut contents generally are not vomited and taken up by the crop. The role of defensins stored in the crop of strictly hematophagous, vessel-feeding flies may not be as important as it is for blood-feeding/pool-feeding and nonblood-feeding flies that put large quantities of both proteinaceous nutrients (such as feces) and surface carbohydrates into the crop. Flies feeding on animal feces or decaying foods consume large numbers of pathogens that enter the crop. Omnivorous plant-feeding flies such as *Drosophila* produce in both the salivary glands and the labellar glands antimicrobials that end up in the crop. Pathogenic microbes are presumably dealt with in the crop, which is their major storage organ for nectar and other free nutrients (32).

## Osmotic Swings in the Hemolymph Lead to Possible Midgut Damage

All animals must avoid significant body fluid osmolarity fluctuations. The storage and gradual release of crop contents into the midgut may be designed to prevent this. One of the first researchers to suggest this was Denisova (15), who stated that the crop prevented what he called "undue dilution of the body fluids." Support for this comes from studies on tsetse in which solutions, not isotonic with the vertebrate blood meal or solutions high in potassium, retarded or prevented crop emptying (67). Nicolson (82) later elaborated on this and supported this function of the crop.

## Drinking Water and Crop Involvement

Flies do not generally imbibe plain water, and if they do, it is in small amounts. This may be because what they normally drink is usually dissolved in water, and water by itself is a poor phagostimulant (16) unless the flies are dehydrated (30). If flies are dehydrated, the tarsal taste threshold to a water response is lowered, whereas the reverse is true for hydrated flies (30). Edgecomb et al. (30) were able to get flies to take 2.8 to 4.0 mg of water into the crop, but this only occurred by stimulating the tarsal receptors with sugar. *P. regina* holds about 2.5 μl of protein diet in its midgut (107), which is probably near the maximum. When *P. regina* was injected with demethylchlordimeform (DCDM), clonidine, or pargyline, it consumed between 11.4 and 14.3 mg of water (76), and the authors suggested that octopamine-type receptors positively modulate the central nervous system, causing the flies to drink water in excess. Unfortunately, the Long & Murdock (76) did not mention where the water went, but it must have gone to the crop, as well as the midgut, because flies injected with only saline increased their weight by about 1.5 mg, and the maximum the midgut holds is 4.0 mg, if their figure is correct. If flies drink only water, it is generally dispatched to the midgut for absorption and normally does not go to the crop (104). For flies, a diet of mostly water has circumvented the need to drink just water. Adult *D. melanogaster* closely regulate the osmolarity of the hemolymph, and dehydrated flies can rehydrate to normal levels by drinking different solutions (i.e., distilled water, a sucrose-salt mixture). On either solution, flies recovered hemolymph values

equal to normal (2). The only mention of where the food went in flies that imbibed was that some flies had "...a largely distended gut." Any overriding mechanism controlling thirst in flies still remains unsolved (5, 16, 100).

**DILPs:** *Drosophila* insulin-like peptides

**Trap-lining:** a behavior in which the fly regurgitates liquids from the crop and drops single droplets in a line or circuitous route

### Regulation of Hemolymph Sugar Concentrations

Recent findings in *D. melanogaster* suggest a direct role for the crop in carbohydrate metabolism. Transgenic ablation and injection experiments demonstrated that the storage and release of cellular carbohydrate reserves are regulated antagonistically by *Drosophila* insulin-like peptides (DILPs) produced in brain medial neurosecretory cells and by AKH produced in the CC, respectively. AKH signaling induces lipolysis and glycogenolysis in the fat body and elevates hemolymph carbohydrate levels (61, 69). Conversely, signaling by one or more of the seven identified DILPs triggers peripheral uptake of circulating carbohydrates (10, 52, 94). These highly conserved signaling systems regulating carbohydrate homeostasis may be extended to modulate crop activities in the Diptera. Although many insects store carbohydrate reserves as fat body glycogen, flies rely heavily on nectar carbohydrates stored in the crop, particularly during the metabolically demanding activity of flight (59). Cao & Brown (12) described DILP immunoreactive axons projecting from DILP-producing brain medial neurosecretory cells branching and running along the crop duct. Lee & Park (69) described AKH immunoreactive processes extending from cells that originate in the CC and running down along the crop duct to the point where the lobes begin (possibly P4, **Figure 1*d***). These authors went on to hypothesized that AKH may trigger crop emptying into the midgut to induce hyperglycemia when hemolymph carbohydrate levels have been lowered. Taken together, the potential systemic effects of DILP and AKH hormonal signaling may be reinforced by a direct release of these signaling molecules as neuromodulators or myoactive peptides where they act on the muscles. In this scenario, insulin signaling may inhibit muscle contractions and prevent further movement of carbohydrate-containing fluids into the midgut while triggering peripheral cells to clear circulating carbohydrates. AKH signaling may have the antagonistic effect of mobilizing cellular carbohydrate reserves and while stimulating crop contractions to move more carbohydrate-rich fluids into the midgut. Presumably, in both studies the axons are located inside the crop nerve bundle and were described previously (109).

## BEHAVIORAL INVOLVEMENT OF THE CROP

Some dipterans usurped through evolution the basic physiological function of the crop and incorporated it into diverse and essential behavioral aspects of their biology. The major group doing this is the fruit flies, several families of which use the contents of the crop as a nuptial gift (**Figure 1*f***). Fruit flies also use the crop to "plant" bacteria on the host plant by trap-lining (**Figure 1*g***) and as a transfer site for a lekking pheromone produced in the salivary glands. Other groups outside of the fruit flies also use crop contents as nuptial gifts; the extent of this behavior in Diptera is unknown (22, 34, 103).

### Sensory Trapping and Nuptial Gifts from the Crop

The importance of a male's crop contents in sensory trapping of the female for mating and of the contributions of this nuptial gift to the female are poorly studied in the Diptera, even though flies provide one of the best examples of mating systems in which males lure nutrient-seeking, sensory-deprived females to feed on the droplet they produce (**Figure 1*f***). Field experiments have not reported whether females will mate with males not producing this droplet. Laboratory experiments

preventing *D. subobscura* males from making the bubble or droplet showed that females still mated with them (103), but whether this occurs in nature is not known.

Vahed (120) makes reference to the adage "all that glitters is not gold." Flies are attracted to glistening objects (65), including honeydew, extrafloral nectar, and pycnidial nectar (24, 89). Additional evidence of visual sensory trapping of flies with glistening objects is found in the carnivorous sundews. Numerous studies have shown that the droplet produced by the males of certain fly species is produced by fluids in the crop and represents a form of regurgitation. We have established that sugars, and in some cases proteinaceous nutrients, are directed to the crop for storage. In addition, we discussed studies showing that enzymes and antibacterial substances are produced in the salivary and labellar glands and end up in the crop. Thus, a droplet of solution from the male's crop, whether maintained on the tip of the male's proboscis and shared with the female via her proboscis in a "kiss" (i.e., mating trophallaxis) (54, 103), dropped as an undisturbed droplet, or whipped up into a froth (64), serves both as a visual sensory trap and as a sensory phagostimulant. The diet of the male and the nuptial gift from the crop, shared with the female during mating trophallaxis, were demonstrated to influence the total number of copula (3). In several cases in which a male shares droplets with a female who consumes them, they may be sharing several of these regurgitated droplets, not just one.

Certain Asteiidae form leks on leaves where the male regurgitates droplets, some of which he shares with the female during copulation. The male also drops many of them onto the leaves and the female consumes them, but only after copulation when she searches the leaves and consumes any remaining droplets (34). Readers are referred to Headrick & Goeden (54) for the most comprehensive treatment of the literature on this topic. Whether crop droplets serve as lekking pheromones in these flies is not known. Starved *D. subobscura* females took significantly more droplets from fed males than from starved males. This indicates that the female somehow assesses the male's nutritional status and that the hypothesis of nutrient deprivation applies (103). The idea that bubbling, or the formation of droplets, helps mix food with salivary gland secretions in the crop was suggested by Hewitt (57). Females might also utilize the bubbling behavior of males as a signal that the male has a certain size crop volume, because it has been shown that only flies having a certain sized crop will bubble (106). Bubble or droplet size has been correlated with larger males in *D. subobscura* (103), and size in some flies influences successful mating (108). Whether bubble sharing commences only after the male sees the female has not been reported. The underlying mechanism eliciting this type of crop regurgitation also remains unknown, especially in those cases in which the males add salivary gland secretions to the bubble and whip it into a nuptial pillar (22) (**Figure 1*f***).

## Pheromone Precursors in the Male Crop

Basic information on the importance of regurgitation and chemical identification of the sugar-based oral secretions from the crop onto plant tissues is lacking. Observations show that male *Anastrepha suspensa* (Loew) deposit oral droplets from the crop onto the underside of leaves. These droplets serve as pheromone lekking attractants for conspecific males and virgin females, and the pheromone is slowly released over several days. Chemical analysis showed that the crop tissue itself contained more of the three terpenoids, suspensolide, β-bisabolene, and α-farnesene, than did the crop contents (78). No TEM of the crop tissue of *A. suspensa* has been reported, and it is possible, but not probable, that the crop tissue has become modified through evolution to produce these chemicals. More likely, as has been demonstrated in other species, these chemicals are found in the highly developed and sexually dimorphic salivary glands of the male only, and these chemicals ultimately end up in the crop during salivation and reingestion (78). Further

studies showed that this sugar-based semiochemical release system is a natural abiotic mechanism for pheromone release dependent on relative humidity fluctuations, paralleling the daily patterns of the flies' reproductive and aggregation activity (125).

**Horizontal gene transfer:** a process whereby genes are transferred from one organism to another via transposable elements and viral infection

## Trap-Lining or Deposition of Bacteria from the Crop onto Plant Surfaces

Most tephritids rely on bacteria in their diet, and the adult crop is an important organ system for this insect/microbe association. Adult flies ingest bacteria into their crops, and these bacteria can provide a sufficient diet for breeding (26). The crop was established as the major storage organ, and it is from the crop that the fly deposits onto leaf surfaces a droplet (as either a single droplet or a trap-line) that contains bacteria. The droplet is produced via regurgitation and probably not by vomiting (26). Some species can deposit up to 120 small droplets in a line and later reingest them (27) (**Figure 1*g***). Most trap-lining species also bubble to concentrate their crop contents (13). Other fruit fly species neither rely on trap-lining nor deposit the droplets onto the substrate; instead, they bubble, presumably to eliminate excess water from the crop (56). This latter group may have a more diverse food source providing nitrogen, such as bird droppings, and has not evolved such a close association with bacteria as those species that trap-line.

When considering the role of the crop in feeding and other functions, one must have a clear understanding of a fly's mouthparts (Do they have labellar hooks or prestomal teeth that rasp the diet, but rely on crop fluids? Where do the salivary glands empty? Where does the crop duct empty?). Some tephritids have a modified and shortened hypopharynx, unlike that found in flies delivering the salivary gland secretions onto the food or substrate. For these fruit flies, the saliva is probably added directly to the food as it is being sucked up. In fact, flies with a shortened hypopharynx (*Euaresta* sp., *Bactrocera*, and *Ceratitis*) use fluids from the crop, rather than salivation, as the main method of ingesting dry and semisolid nutrients and as a way to wash off substances clinging to the micropseudotrachea (13). In the fruit flies *Bactrocera tryoni* (Froggatt), *B. jarvisi* (Tryon), *B. cacuminata* (Hering), and *B. cucumis* (French), if adult flies are dehydrated and without liquid in their crop, they are unable to ingest dry or semisolid nutrients (123). Thus, the crop, mouthparts, salivary glands, and feeding strategies of flies are strongly related to one another.

## CONCLUSIONS

Sugar feeding is essential to adult dipterans and the diverticulated crop contributed to this ability. The Diptera are the major insect vectors of pathogens of humans and of domestic and wild animals; they are also a major nuisance to warm-blooded animals. We have presented the various ways in which the crop is used in flies, all of which contributed to dipteran diversity and success. At the same time, the crop is a major reservoir or storage area for pathogens where gene exchange leading to horizontal gene transfer of antibiotic resistance takes place (1, 87). Despite the importance of the dipteran crop to flies and to humans, relatively little is known about how this important organ is regulated regarding nutrient uptake and expulsion of its contents.

### SUMMARY POINTS

1. The diverticulated crop is a key synapomorphy allowing for the tremendous success of the liquid-feeding Diptera.
2. The dipteran crop can be a major storage site for carbohydrate- and protein-rich foods, as well as defensins.

3. Carbohydrate digestion is initiated in the crop, largely due to the action of salivary gland enzymes.
4. The crop is under complex neural and neurohormonal control and is coupled directly to nutrient homeostasis mechanisms.
5. The crop has taken on unique roles different from nutrient storage in various dipteran lineages.
6. The crop is an important organ for the transmission of dipteran-borne pathogens.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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