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LETTER TO THE EDITOR

Beadex affects gastric emptying in *Drosophila*

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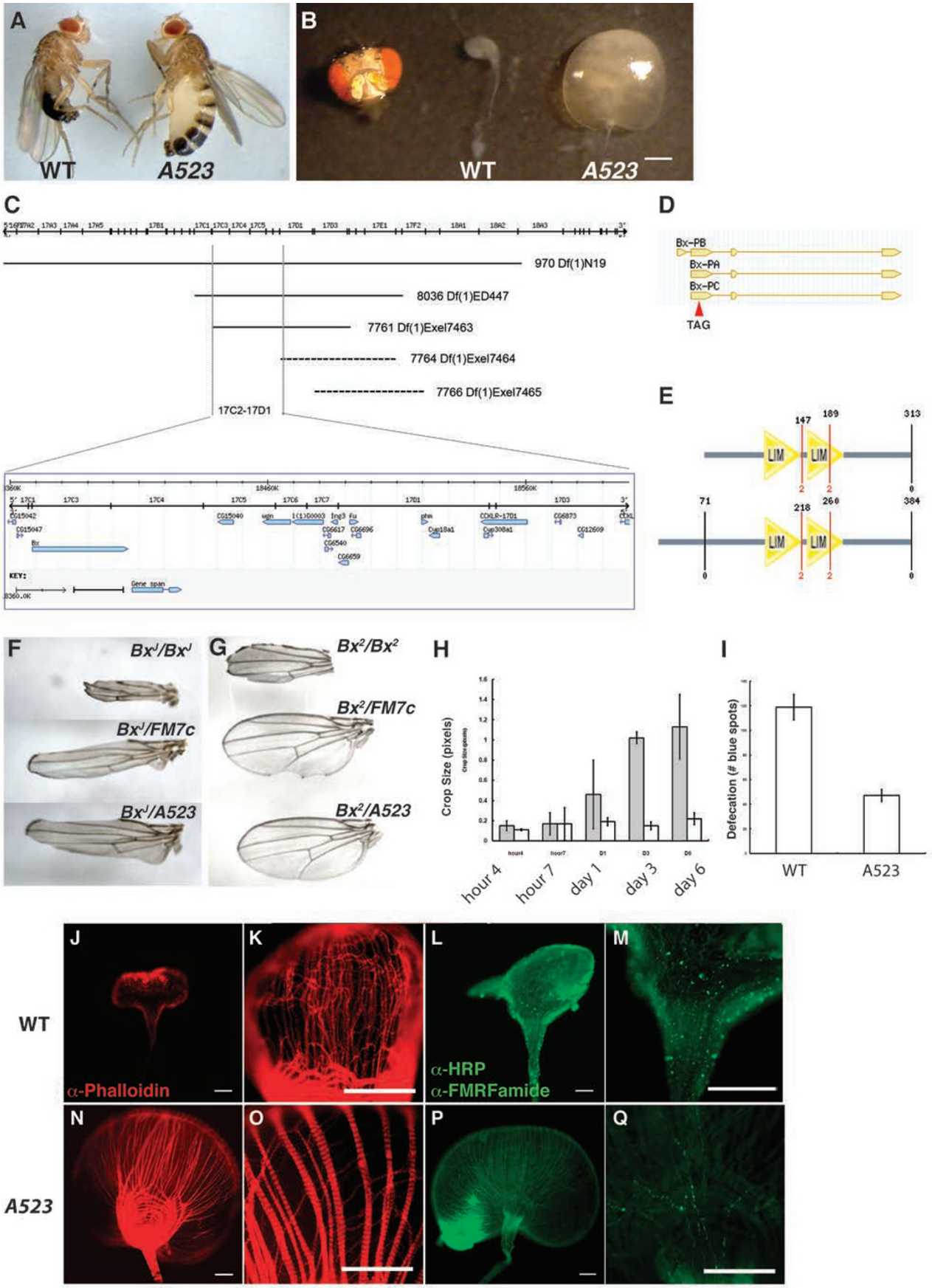
Dear Editor,

The ingestion and digestion of food is a universal aspect of animal growth and survival and is important for our understanding of obesity and diabetes. All animals have a digestive system that comprises of an upper gastrointestinal tract mainly for the ingestion and storage of food, and a lower gastrointestinal tract or the gut for digestion and absorption. When food is ingested, peristaltic contraction is initiated and directs the bolus through the esophagus toward the stomach or an equivalent structure [1]. In addition to serving as the major site for digestion, the stomach acts as a reservoir to store food. This adaptation may have significance for the survival of many animals in which the immediate access to the infrequent availability of food necessitates large meal consumptions that can then be slowly digested or utilized at a later time. Once the food is triturated in the stomach, it is released into the lower gut where the main function of digestion and absorption of nutrients is carried out. To ensure the efficient extraction of nutrients in the lower gut, gastric emptying of liquefied food from the stomach into the lower gastrointestinal tract must be regulated and coordinated with the status of digestion and absorption. The release of nutrients from early phase of digestion such as complex carbohydrates, fats, and proteins, provides an inhibitory feedback signal to slow the rate of gastric emptying [1]. In mammals, it is known that these nutrient signals in turn regulate the secretion of gastrointestinal hormones and peptides to modulate gastric emptying [2, 3]. This negative feedback allows ample time for the lower gut to complete the process of digestion and absorption. A major question, however remains to be unclear is how the completion of digestion and absorption in the gut is communicated to the upper gastrointestinal tract to stimulate gastric emptying. It is possible that digestion product(s) associated with the late stage of digestion and absorption may serve as the trigger for the process. To better understand these processes, we turn to the fly as a genetically tractable model system to dissect the function and physiology of digestion and absorption. Both flies and humans have been shown to share surprising conservation in major cellular and molecular pathways, particularly with many metabolic pathways in common [4]. Fundamentally, they also share similarities in their behavioral and physiological responses to eating. First, numerous studies in insects and *Drosophila* have shown that they possess anatomically analogous gastrointestinal structures that carry out similar functions to those in higher organisms. For example, the *Drosophila* foregut or the esophagus is connected to an expandable sac-like structure called the crop, which has been thought to serve as a storage organ for ingested food (Figure 1B). Enveloped by visceral muscles that behave similarly to vertebrate smooth muscle, the crop is capable of contraction and peristaltic movements reminiscent of the human stomach. Similar to the small intestine in mammals, the fly midgut is the site of digestion and absorption, which is connected with the hindgut, a structure equivalent to the colon in mammals for water absorption. Second, eating behavior and postprandial gastrointestinal functions in *Drosophila* have been shown to be influenced by both endocrine and neural pathways, which have homologous components that function similarly in humans [5]. However, the underlying cellular and molecular mechanism regulating gastrointestinal functions is largely unknown.

We report here a genetic screen in *Drosophila* for mutants that are defective in gastric emptying. We reasoned that defects in gastric motor or sensory function or the stimulus signaling, as in humans, would lead to a dysfunction in gastric emptying. An easily identifiable phenotype would be an enlarged abdomen as a result of the accumulation of food in the crop and the failure to empty into the midgut. Indeed, a recent characterization of the *drop-dead* (*drd*) mutant with a bloated abdomen phenotype revealed that it has defective gut function in crop emptying and associated reduced rate of defecation [6]. However, since crop contraction was not affected, it was unclear what the underlying basis for phenotype is. In our screen we have identified mutants with bloated abdomens.

Here we focus on the initial characterization of one of the candidate mutants, *A523*, which exhibits abnormal gastric emptying from the crop (Figure 1A and 1B). Mapping analysis placed the mutation on the X chromosome to a region defined by cytological bands 17C2-


A
WT
A523
B
WT
A523
C
970 Df(1)N19
8036 Df(1)ED447
7761 Df(1)Exel7463
7764 Df(1)Exel7464
7766 Df(1)Exel7465
17C2-17D1
KEY:
Gene span
D
Bx-PB
Bx-PA
Bx-PC
TAG
E
147
189
313
LIM
LIM
71
218
260
384
F
Bx^J/Bx^J
Bx^J/FM7c
Bx^J/A523
G
Bx^2/Bx^2
Bx^2/FM7c
Bx^2/A523
H
Crop Size (pixels)
hour 4
hour 7
day 1
day 3
day 6
I
Defecation (# blue spots)
WT
A523
WT
A523
J
K
L
M
α-Phalloidin
α-HRP
α-FMRFamide
N
O
P
Q

17D1 (Figure 1C). Sequencing of the mutant genomic DNA revealed a single point mutation that introduces an early termination codon in the coding sequence of the *Beadex* (*Bx*) gene, which encodes the dLMO transcription factor containing two conserved LIM domains [7, 8] (Figure 1D and 1E). The gene is known to be involved in a wide range of development and behavior phenotypes [7, 8]. To demonstrate that *A523* is indeed a loss-of-function allele of *Bx*, we show that the *A523* mutation was able to effectively suppress the dominant wing phenotype of *Beadex* mutants, which are gain-of-function alleles of the gene [8, 9] (Figure 1F and 1G).

The *A523* mutant adults acquire a bloated abdomen as a result of progressive food accumulation in the crop (Figure 1H). This phenotype is apparent one day after eclosion. The enlarged crop phenotype is not due to progressive excessive food intake, but rather correlated with a significant reduction in defecation indicating a defect in gastric emptying (Figure 1I and data not shown). To begin understanding the basis for these defects, we examined the crop structure for any abnormality in muscle function. Phalloidin staining, which highlights muscle fibers, show that the muscles ensheathing the crop differentiate and pattern normally (Figure 1J, 1K, 1N, 1O). Although the crop is fully enlarged for a long period of time, there is no indication of the loss of muscle integrity such as broken fibers.

It is known that the crop contraction is affected by numerous neuropeptides secreted by innervating neurons. We asked whether the *A523* crop phenotype arises as a result of a defect in such signaling. Staining with anti-HRP, which outline the neurons of the crop, and also with anti-FMRFamide, which detects the expression of the crop contraction-inducing neuropeptide FMRFamide, revealed that the neural pathway innervating the crop, at least with respect to FMRFamide, appears to be fully present (Figure 1L, 1M, 1P, 1Q).

The *Drosophila* esophagus is directly connected to the proventriculus of the midgut while the crop is connected to the esophagus just anterior to the proventriculus. It is not known whether ingested food directly passes into the midgut from the esophagus and excess amount of ingested food is diverted into the crop for storage. Alternatively, all ingested food has to initially enter the crop and in subsequently pumped into the midgut for digestion. The enlarged crop phenotype observed in the *Bx* and *drd* mutants could be due to excessive diversion of food into the crop for storage or a defect in the transfer of food from the crop to the midgut. To distinguish these possibilities, we examined the passage of food through the digestive system by following labeled ingested food in adult animals (Supplementary information, Data S1). Our results show that ingested food does not enter directly into the midgut, but rather is initially diverted to the crop during ingestion and subsequently pumped into the midgut after ingestion. This suggests that the crop plays a critical role in regulating food passaging. The fact that *A523* and *drd* mutants are defective in transferring food out of the crop and show reduced fecal deposition provides further support for this role and indicates that these genes are important for this process [6].

In conclusion, our genetic screen demonstrates the feasibility of using a fly model to identify candidate mutants that affect gastrointestinal function. Furthermore, the crop plays a critical role in gastric emptying. Proteins containing LIM domains have been found to play important roles in many biological processes such as cytoskeleton organization, cell specification and differentiation, as well as organ development and pathological functions [10]. Further studies of *Bx* using tissue-specific Gal4 drivers for different parts of the digestive and nervous systems will hopefully shed light on the mechanism of its physiological regulation of processes such as gastric emptying.

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Figure 1 Identification and characterization of a mutant defective in gastric emptying. **(A)** Adult *A523* mutant displayed a bloated abdomen. Animals are 3-4 days old, except where indicated. **(B)** Dissection revealed an enlarged crop in the mutant as compared to wild type with size reference to the adult head. **(C)** Deficiencies mapping uncovered the mutation to cytological region 17C2-17D1. **(D)** Sequencing revealed a single point mutation in the *dLMO* gene carried by the *A523* mutant. **(E)** Alternatively, spliced dLMO transcripts encoded two proteins with the same C-terminal regions containing two LIM domains. **(F**, **G)** Bx^J and Bx^2 wing phenotypes can be suppressed in trans-heterozygotes with *A523*. **(H)** *A523* mutants show a progressively enlarged crop size apparent after 1 day post eclosion. **(I)** *A523* mutants show a reduction in fecal deposition. **(J**-**O)** Phalloidin stainings of the crops of wild type **(J**-**K)** and *A523* mutant **(N**-**O)**. **(L**-**Q)** Anti-HRP and anti-FMRFamide stainings to highlight neurons and neuropeptide-expressing cells, respectively. **(L**-**M)** wild type. **(P**-**Q)** *A523* mutant. The signals are detected in both wild type and mutant crops, indicating that FMRFamide secretion is present in the mutant.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)