

Abnormal Proventriculus in Bumble Bee Males

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Abstract: Females social insects are widely investigated, while males are often neglected. Previous work on the morphology of the bumble bee male alimentary tract has described the presence of swollen proventriculi as a character with taxonomic value. New observations suggest that the abnormal proventriculus might be an abnormal condition. Here, I identify a potential health issue in bumble bee (Hymenoptera: Apidae: *Bombus*) male alimentary tracts that consists of a swollen and strongly melanized proventriculus, thereby preventing the normal passage of food through the esophageal valve. Males from three bumble bee species exhibited the abnormal proventriculus with an overall prevalence of 31.1% across two years. This finding challenges previous research that suggested the swollen and melanized proventriculus of *Bombus* males as a taxonomic character.

Keywords: *Bombus* sp.; swollen proventriculus; health condition; taxonomy; social insects; male

1. Introduction

The organization of the alimentary tract of bumble bees consists of the fore gut (pharynx, esophagus, honey stomach, proventriculus, esophageal valve, and salivary glands), the gut (ventriculus), and the hind gut (Malpighian tubules, pyloric valve, ileum, colon, and rectum) [1]. The proventriculus and the esophageal valve form a valve-like structure that clearly separates the fore gut and the mid gut. The anterior end of the proventriculus is projected up into the posterior end of the honey stomach and exhibits four triangular lobes of proventricular wall tissue [2,3]. The presence of chitinous spines in the inner part of each lobe probably prevents the pollen from returning back into the esophagus. The proventriculus plays a key role in managing the flux of food, and it is like a force pump for the entire alimentary tract. Therefore, injuries or diseases altering the correct functioning of the proventriculus will affect the uptake of nutrients in the posterior parts of the alimentary canal; hence, they will have an impact on the survival of the animal. In flea species (*Pulex* sp.), a proventricular block can happen while the animal is feeding upon the blood of a host that is infected with the plague bacilli, due to the proliferation of the bacteria; a process that is temperature-dependent [4]. Macfarlane [5] studied 15 species of *Bombus* and two of *Psithyrus* and found that the proventriculus of *Bombus* males had four orange-brown projections, while all of the females or males of the other species had a normal, unswollen proventriculus; thus, suggesting the role of this character in the taxonomy of the clade.

Here, I report on a new, potentially male-specific health condition affecting the proventricular valve of bumble bees, and therefore, reject the use of the swollen *Bombus* male's proventriculus as a taxonomic character.

2. Materials and Methods

I examined two sets of wild bumble bees from different species that are present in Ireland: 161 samples from Howth (between 30 July and 23 August 2003), and 189 samples from Irishtown Nature Park (between 5 July and 13 August 2004). All of the animals were frozen to death and dissected under a dissecting microscope at $\times 10$ optics with $\times 0.1$ – 5.0 on the magnifying knob, using micro-forceps (Dumont 5) and spring-scissors (InterFocus,



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F03). The abdomen was separated from the thorax, and it was fixed dorsally to a wax-bed with entomological pins. The first tergite was cut following the ventral curve and then, the next cut ran perpendicularly to the first incision and either side was cut, thus leaving the last tergite untouched. The animals were examined within weeks after the sampling and preserved at $-80\text{ }^{\circ}\text{C}$. This work complied with the laws governing animal research in Ireland.

3. Results

From a total of the 161 bumble bees that were studied from Howth, I found 42 males and 119 females. All of the females exhibited normal guts with an unswollen proventriculus. I found, however, that the proventriculus of more than 40% of the males were abnormal when compared with those of the other animals. These latter proventriculi had the four triangular-shaped proventricular lobes that were abnormally swollen and showed strongly melanized semispherical-like shapes (Figure 1A–D). In Irishtown, 64 out of the 189 sampled animals were males among the six different species that were present. The prevalence of males with abnormal proventriculus varied between the sites and across the species (Table 1).

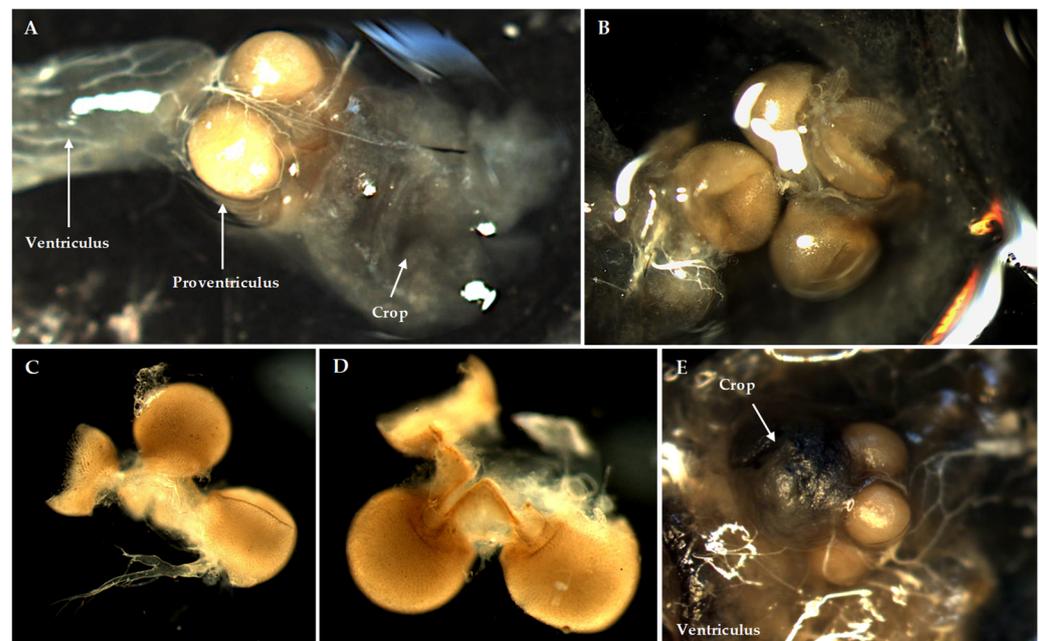


Figure 1. Abnormal male proventriculus. (A) Part of the alimentary tract of one *B. terrestris* male, showing the ventriculus, the abnormal proventriculus, and the crop or honey stomach. (B–D) Details of the abnormal proventriculus showing a high degree of melanization and abnormal growth; $400\times$ (E) Crop containing plenty of rotten pollen, which was partially cemented and unable to pass through the proventriculus due to the deformation of its four lobes. Pictures recorded using Q IMAGING—Micropublisher (using IP Lab, Mac OS X, 10.3), and corrected using GIMP 2.10 (www.gimp.org, accessed on 13 September 2022). A normal alimentary tract of a bumble bee can be found in Zhang & Zheng [6].

In some of the males, the four lobes of the proventriculus were so swollen that it prevented the passage of pollen through the valve, and the honey stomachs were extremely distended due to there being great quantities of pollen. Moreover, in those cases, parts of the contents of the honey stomach consisted of cemented pollen, which showed clearly signs of decay in several animals (Figure 1E).

Table 1. Proportion of bumble bee males with an abnormal proventriculus. Total and abnormal males found for each species, and percentage of males with deformed proventriculus.

Howth 2003			
Species	Total males	Abnormal males	%
<i>B. terrestris</i>	32	16	50
<i>B. lucorum</i>	8	1	12.5
<i>B. pascuorum</i>	2	1	50
TOTAL	42	18	~43
Irishtown 2004			
Species	Total males	Abnormal males	%
<i>B. terrestris</i>	16	12	75
<i>B. lucorum</i>	4	3	75
<i>B. pascuorum</i>	20	0	0
<i>B. muscorum</i>	3	0	0
<i>B. lapidarius</i>	17	0	0
<i>B. pratorum</i>	4	0	0
TOTAL	64	15	~23

4. Discussion

Here, I provide the first description of a potential new disease that is specific to male bumble bees. From the 350 studied animals, none of the 244 females showed signs of swollen guts. From the 106 males that were collected in both places, 33 exhibited the deformed guts. I only found this abnormal condition in the males from the three species: *B. terrestris*, *B. lucorum* and, only in Howth, *B. pascuorum*. The prevalence of it was higher in Howth than it was in Irishtown, but I conducted the sampling over two different years, and thus, both values of this prevalence cannot be compared. This abnormal condition, however, was not found in any other species possibly due to the small sample size. The male abnormal proventriculus that is reported here strongly agrees with the description that is provided by Macfarlane [5], but it does not support its value as taxonomic character. Therefore, both the incidence and prevalence of this condition are highly heterogeneous at the geographical, temporal, and species levels. Female bumble bees have been extensively and widely studied, while males have been neglected [7]; thus, it is very likely that this is a sex-specific condition. The latter implies that either the females never face the cause that brings about this condition, or if they do, they always resist it. Thus, I can venture some possible sources of variation between the sexes: (i) males exhibit differences in behavior and lifestyle when they are compared to females, which may expose them to higher doses of toxicants and microbiological threats [8]; (ii) males exhibit more variability than females due to their haploid condition: while it has been shown that a haploid condition might purge male animals with certain recessive alleles [9], males are phenotypically more variable than the females [10], and so the development of an abnormal proventriculus might be due this variability; (iii) changes in the composition of the gut microbiome [6,11,12]. I can think of several potential non-exclusive causes for the occurrence of the swelling and the melanization of the four proventricular lobes in males: (i) A physical injury in the esophageal valve, caused either by an object or a pathogen, followed by an immune reaction that triggered the melanization of the proventriculus [13,14], maybe after an inflammatory process [12]; (ii) Some chemical compound that is present in the food (e.g., pollen defenses) or the environment (pesticides or herbicides) that triggers the abnormal growth and melanization of the lobes. However, while there exists a positive effect of pollen defenses and bumble bee gut melanization, pesticides and herbicides seem to inhibit melanization in insects [15–17]. (iii) The abnormal proventriculus might also be the result of a sex-specific pathogen or a parasite that went unnoticed because males do not return to the nest, thus preventing its transmission within the colony; (iv) Another explanation

is that a common intracellular endosymbiont that is present in workers, *Wolbachia* sp., behaves differently in males when it is infecting the proventriculus [18]. Commensal or saprophytic microbes that are inhabiting the alimentary tract of the insects do not cause damage to their hosts, but some viruses are known to induce the hypertrophy or sloughing of midgut cells in lepidopteran larvae or diarrhea [19]. Moreover, in the proventriculus of tse-tse flies, a microbial challenge induces the transcription of antimicrobial peptides, reactive nitrogen intermediate (RNI) nitric oxide synthase (NOS), and accumulates reactive oxygen intermediate (ROI) and hydrogen peroxide (H₂O₂) [20]. In addition, the latter hypotheses could only work if there exists behavioral or physiological differences between males and females. All hypothesis, however, remain to be investigated. The males of the model organism *B. terrestris* exhibited the highest occurrence of it, so they are the opportune system to be used to design experiments under controlled conditions to test some of the proposed hypotheses; the changes in gut microbiome or the presence of pathogens can be explored by a metagenomic analysis of the wild males. Whatever their provenance, the swollen and melanized lobes prevent the normal passage of food through the esophageal valve in the studied animals, thus, preventing the nutrition of the animal.

5. Conclusions

The swollen proventriculus that presents in some bumble bee males of different species is an abnormal condition that prevents the passage of food with poor or no taxonomic value.

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