D-37: Digestion of blood in vector mosquitoes *Culex quinquefasciatus* and *Anopheles tessellatus*

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The intake of a blood meal provides the nutritional requirements for egg development in female mosquitoes and also serves as a point of infection with pathogens. Ingestion of the blood meal leads to a series of physiological changes in the mosquito that trigger the synthesis and release of digestive enzymes and the stimulation of egg development. Ingested proteins are digested in the midgut and the major protease synthesized in the mosquito gut is trypsin; in addition chymotrypsin and aminopeptidase are also present.

This investigation describes blood digestion and egg development in the malaria vector *Anopheles tessellatus* and the filariasis vector *Culex quinquefasciatus* mosquitoes.

An. tessellatus and *Cx. quinquefasciatus* mosquitoes were obtained from laboratory colonies. Adult mosquitoes were maintained on 10% glucose solution. Blood meals were provided to 3-4 days old An. tessellates mosquitoes by feeding either directly on a restrained rabbit or *in vitro* through a glass membrane feeder. *Cx. quinquefasciatus* were fed directly on a rabbit or on a cotton wool pledget. Blood fed mosquitoes were separated and kept in cups. They were frozen at defined time points and midguts dissected to evaluate egg development. Midguts were homogenised and used with the substrates Nα-DL-Arginine p-Nitro Anilide Hydrochloride (BApNA) and N-Benzoyl-L-Tyrosine p-Nitro Anilide (BTpPNA) for trypsin and chymotrypsin; optical densities were measured on a spectrophotometer.

The Soybean trypsin inhibitor and leupeptin were added to the blood meal and presented to the mosquitoes through a membrane feeder. The influence on trypsin synthesis and egg development was investigated as described above.

In blood fed *An.tessellatus* mosquitoes activity reached a peak (11 -14 BApNA units) 18-24 h post blood meal. However several minor peaks were observed suggesting several waves of trypsin production. In *Cx. quinquefasciatus* mosquitoes, trypsin activity increased and reached a peak (32 BApNA units) at 24-26 h but no minor peaks were observed. Trypsin activity was higher in *Cx. quinquefasciatus* than in *An. tessellatus* mosquitoes, which may be related to the larger average blood meal size of *Cx. quinquefasciatus*.

Chymotrypsin activity was slightly elevated during the blood meal digestion in *An. tessellatus* mosquitoes. Addition of trypsin inhibitors, soybean trypsin inhibitor and leupeptin lowered trypsin activity and delayed peak activity. Egg development was poor in trypsin inhibitor fed mosquitoes where no egg development was observed at 1.5 mg/ml level of SBTI and only 3% of mosquitoes developed eggs at 0.5 mg/ml SBTI level (control 60%).

Trypsin activity in *An. tessellatus* reach to a peak (18-24 h) earlier than *Cx. quinquefasciatus* (24-26 h). Rapid digestion of blood meal and egg production in *An. tessellatus* may lead to a shorter life cycle in anophelines which is advantageous in an environment where breeding grounds, e.g. temporary rain pools exist only for a very short time. Trypsin inhibitors can be used to delay and/or lower the peak of trypsin and therefore may be used to investigate the role of trypsin in the transmission of malaria and filarial parasites to the mosquito vectors.