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Gene targeting for better flea control

External parasites represent a considerable nuisance and cause of suffering and economic losses in companion animals, livestock and humans. Among these, the cat flea *Ctenocephalides felis* is a widespread pest across temperate and tropical areas of the world with particular importance in domestic pets.

The cat flea is the dominant flea species infesting both dogs and cats, and as a blood feeding parasite is additionally capable of causing harm by acting as a disease vector. This includes transmission of the bacterial diseases flea-borne spotted fever (*Rickettsia felis*) and cat-scratch disease (*Bartonella henselae*). These fleas are also intermediate hosts of the intestinal tapeworm *Dipylidium caninum*, which is spread to dogs and cats via ingestion of infected fleas. Besides its roles in the spread of infectious disease, cat fleas cause skin disease (flea allergy dermatitis) in animals, particularly dogs, that are hypersensitive to flea bites.

Current flea control measures are based on chemical agents, which include insecticides and insect growth regulators. Further improvements in flea control will be greatly aided by detailed knowledge of flea physiology at a molecular level. In the case of the cat flea, this knowledge remains fragmentary. Identification of genes expressed in the flea in response to feeding is of key importance to elucidate the mechanisms that permit successful feeding, digestion, immune defence and reproduction. Several feeding-specific genes have been isolated from the flea to date, however many other components involved in the physiology of feeding remain to be identified.

Methods and results

We obtained genetic material (RNA) from whole cat fleas before and 1 day after a blood meal. We compared these samples by the molecular technique of suppression subtraction hybridisation, which was designed to identify transcripts that are switched on or upregulated in response to feeding. Enriched DNA bands detected after agarose gel electrophoresis (Figure 1), were cloned and subjected to sequential hybridisation with complementary DNA (cDNA) from fed and unfed fleas as probes in order to confirm differential expression.

Nine cDNA clones (B2, S5, S16, B43, S49, B52, S58, S61, B68), confirmed to be independent transcripts, were selected for sequence analysis following 5′ and 3′ RACE (Table 1). Two (B2 and S16) were found to encode digestive enzymes (chymotrypsin-like serine proteases), while four others appeared to be molecules with various protective roles, namely a serine protease inhibitor (S49), a novel peptidoglycan recognition protein (B52), and two related novel mucins (S58 and B68).

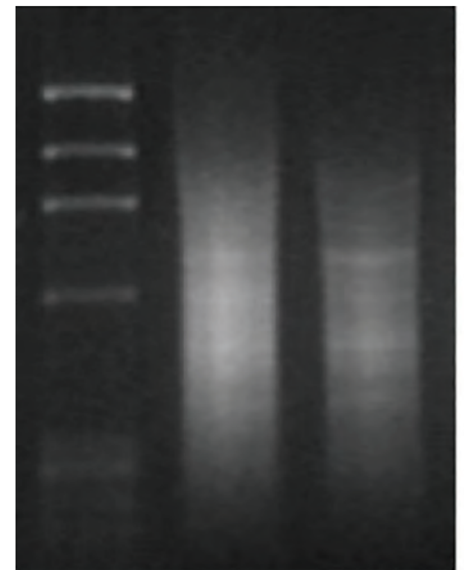


FIGURE 1 Agarose gel electrophoresis showing cDNA products associated with feeding by suppression subtractive hybridization

The remaining three genes encoded a novel protein of unknown function (S5), a topoisomerase II enzyme associated with DNA replication (B43) and a glycophosphatidylinositol (GPI) mannosyltransferase 2, an enzyme involved in the biosynthesis of the GPI anchor for the membrane attachment of GPI-anchored proteins (S61).

TABLE 1 Flea genes associated with feeding

Gene ID	Gene name	Biological function
B2	Chymotrypsin-like serine protease	Proteolytic digestive enzyme
S5	Novel	-
S16	Chymotrypsin-like serine protease	Proteolytic digestive enzyme
B43	DNA topoisomerase II	DNA replication
S49	Serine protease inhibitor (Serpin) 3	Serine protease regulator
B52	Peptidoglycan recognition protein LB-like	Innate immunity pattern recognition receptor
S58	Mucin-like	Protection and lubrication of epithelial linings
S61	GPI mannosyltransferase 2	GPI-mediated membrane attachment of GPI-anchored proteins
B68	Mucin-like	Protection and lubrication of epithelial linings

Differential expression of these nine genes was confirmed by polymerase chain reaction (PCR) using RNA from fed and unfed whole fleas, midguts and carcass. A majority of the transcripts (6/9) showed higher expression in both flea midgut and carcass, while two (B2 and B52) showed differential expression that was restricted to flea gut only (Figure 2).

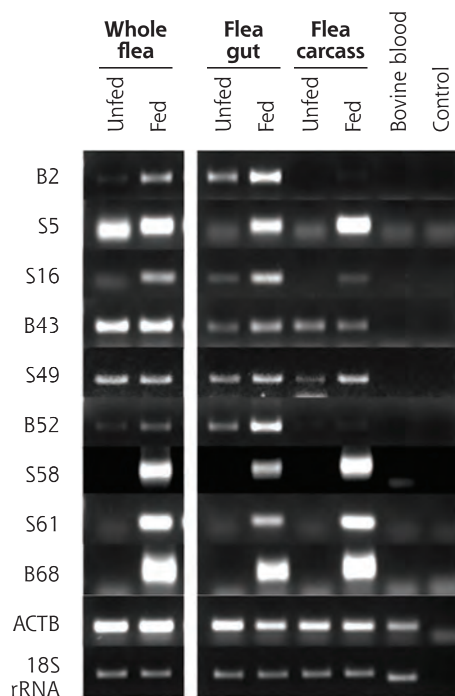


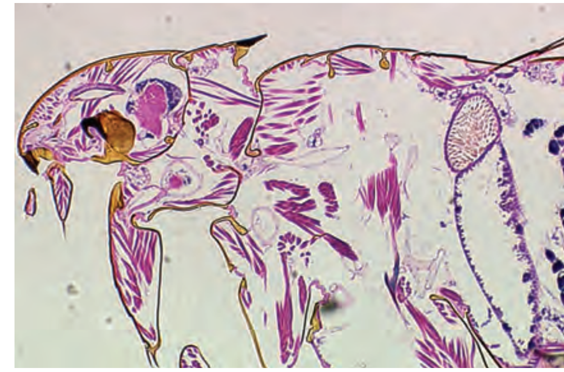
FIGURE 2 Confirmation of differential gene expression between unfed and fed cat fleas by PCR

A final transcript (B43) appeared to show higher expression in fed midgut but lower expression in fed carcass. Thus, all nine fully characterised gene sequences were confirmed to be differentially expressed in fed flea tissues and appear to be modulated by blood feeding.

Conclusions and recommendations

The identification of genes that are switched on by blood feeding is a key step in understanding flea physiology and flea interactions with their hosts, as well as the transmission of flea-borne diseases. Such molecules may also have practical use, representing potential targets for the development of new means of controlling fleas via either chemical or vaccine approaches.

Our results provide a first step in terms of gaining a molecular insight into the physiology of flea feeding. The protein products of the genes identified may play important roles during flea feeding in terms of blood meal digestion, gut growth/repair and protection from infection and feeding-associated stresses. This may ultimately open new avenues for dealing with this ubiquitous parasite and pest. ■



TOP: Histological cross-section of the head and thorax of an adult cat flea.

ABOVE: Flea Incubating Deluxe Oven (FIDO).

More information

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References

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