Understanding the biology and control of the poultry red mite Dermanyssus gallinae: a review

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Understanding the Biology & Control of the Poultry Red Mite,

Dermanyssus gallinae

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Abstract

Dermanyssus gallinae, the poultry red mite (PRM), is a blood feeding ectoparasite capable of causing pathology in birds, amongst other animals. It is an increasingly important pathogen in egg-layers and responsible for substantial economic losses to the poultry industry worldwide. Even though PRM poses a serious problem, very little is known about the basic biology of the mite. Here we review the current body of literature describing red mite biology and discuss how this has been, or could be, used to develop methods to control PRM infestations. We focus primarily on the PRM digestive system, salivary glands, nervous system and exoskeleton and also explore areas of PRM biology which have to date received little or no study but have the potential to offer new control targets.

Keywords: Dermanyssus gallinae, poultry red mite, biology, anatomy, control, mode of action

1. Introduction

Dermanyssus gallinae, the Poultry Red Mite (PRM), belongs to the Order

Mesostigmata which incorporates many mite species that vary considerably in

morphology and behaviour. Many species are phytophagous, saprophagous or

predatory free living species (Koehler, 1999; Gerson *et al.*, 2008) whilst others,

including PRM, have obligatory parasitic behaviour.

PRM is a haematophagous ectoparasite of poultry and wild birds (Kristofík *et al.*,

1996; Brannstrom *et al.*, 2008), requiring blood meals to develop into the last 3

subsequent stages of its life cycle as well as for development of eggs during

oviposition (See figure 1). Predominately females feed on blood several times during

their lifetime though it has been reported that males may blood feed intermittently

(Chauve, 1998). Whilst PRM feeds primarily on birds, it is cosmopolitan in its choice

of host and has been reported to be capable of feeding on rodents (Bakr *et al.*, 1995;

Lucky *et al.*, 2001; Abd El-Halim *et al.*, 2009) and humans (Beck, 1999; Rosen *et al.*,

2002; Bellanger *et al.*, 2008; Collgros *et al.*, 2013;) though these are most likely

accidental hosts and do not sustain a complete PRM life cycle. PRM has been

46   implicated as a transmission vector for several significant animal pathogens,

47   including some that are zoonotic. PRM-mediated transmission between hens has

48   been shown directly for *Borrelia anserine*, fowl poxvirus and eastern equine

49   encephalitis virus (Chamberlain & Sikes, 1955; Shirinov *et al.*, 1972; De Luna *et al.*,

50   2008; Valiente Moro *et al.*, 2009). The transmission of *Salmonella spp.* between

51   birds by PRM has also been demonstrated and moreover the bacteria can be

52   transmitted by mites transovarially to their progeny, rendering PRM a potential

53   reservoir for zoonotic salmonellosis (Valiente Moro *et al.*, 2007). Human cases of

54   salmonellosis have been significantly reduced in recent decades however there is

55   still an industry-wide requirement for safer and better defined vaccines against

56   salmonellosis (Desin *et al.*, 2013) .The potential of *D. gallinae* to harbour and

57   transmit pathogens therefore appears to be an important and emerging problem.

58   Pathology due to PRM in parasitized birds is variable depending on infestation rates.

59   Symptoms from host birds most notably include a decline in general bird health due

60   to lack of sleep and increased self-pecking (Kilpinen *et al.*, 2005). Severe PRM

61   infestations can lead to more serious effects such as cannibalism, anaemia and in

62   some cases even bird death (Chauve, 1998; Kilpinen, et al., 2005). The most

63   economically damaging symptom of PRM infestations is the reduction in egg laying

64   amongst hens as well as a decline in egg quality (Chauve, 1998; Cosoroaba, 2001).

65   Many controls against PRM, such as the use of chemical acaricides and silica dusts,

66   are often sold as broad spectrum substances for controlling a range of farmyard and

67   domestic pests. Reports of PRM resistance to acaricidal drugs containing amitraz,

68   carbaryl and permethrin (Zeman & Zelezny, 1985; Beugnet *et al.*, 1997; Marangi *et

69   al.*, 2009), allied with genetic variation between red mite populations (Brannstrom, *et

70   al.*, 2008; Potenza *et al.*, 2009; Roy & Buronfosse, 2011) suggest there is an urgent

71   requirement for research to uncover more specific control strategies. Detailed

72   knowledge of *D. gallinae* biology and behaviour is comparatively underrepresented

73   in the literature given its commercial impact; this was estimated, for instance, in 2005

74   to cause €130 million per annum economic loss in Europe alone (Van Emous, 2005).

75   Here we present a brief overview of the basic understanding of PRM biology with

76   specific regard to how this relates to current and potential future controls and their

77   modes of action. We provide microscopic imagery of internal morphology, currently

78 lacking in the existing literature, and discuss the types of control that target PRM

79 systems at a cellular and systematic level. It seems increasingly likely that control of

80 PRM will require the application of integrated approaches, a concept we discuss

81 against the backdrop of current ineffectiveness of the existing standalone controls.

82 PRM is yet to be managed efficiently in large scale commercial farming facilities,

83 which leaves an open platform for the introduction of a range of control options and

84 potential for a standardised integrated control management.

85

2. External Morphology

D. gallinae thrives in environments of high (at least 70%) humidity whereas it does poorly in arid conditions because it cannot fully retain moisture (Nordenfors *et al.*, 1999) despite being externally protected by an exoskeleton (see Di Palma *et al* (2012) for detailed diagrams). A dorsal exoskeleton shield covers the length of the idiosoma (body) and is not gender specific. Ventrally however, females present two separate shields; a genitoventral shield spanning posteriorly from leg pairing II and a smaller, more rounded anal shield. Males possess a single, smaller ventral shield comprised of a seemingly fused joining of the genitoventral and anal shields (Di Palma, *et al.*, 2012).

The exoskeleton of acari is made of chitin, a tough and resilient polymer. In an unmodified state, often seen in the larval stage, chitin is translucent and comparatively flexible. Hormones secreted through pores trigger the polymerisation of chitin which is mixed with various protein families and phenolic compounds creating a sclerotized layer. The sclerotized cuticle offers a stiff layer which defines the mite's body shape, aids with muscle attachment and limits water loss (Evans & Till, 1979; Hackman, 1982). Sclerotized cuticle can be identified by a

103   brown/yellowish area, often covering the whole of the outer adult body and is

104   replaced during each moulting stage as it cannot be extended during mite growth.

105   The outer part of the mite exoskeleton, known as the epicuticle, consists of a layer of

106   wax which further limits water loss, and a cement layer which protects the cuticle

107   from external abrasion. Red mite controls, such as silica dust (Maurer & Perler,

108   2006) and diatomaceous earth powder (Kilpinen & Steenberg, 2009), seek to dry out

109   these outer layers and kill PRM through desiccation. Lipid removal through

110   adsorption is thought to be due to the surface migration of fatty molecules into the

111   hollow crystalline structure of the dust particles (Ebeling, 1971) which also interrupt

112   the lipid layers through physical sheering (Vincent *et al.*, 2003). These inert dust

113   particles act via a chemically neutral mechanism and are not associated with any

114   forms of resistance to mite controls, however their use can be limited by

115   environmental conditions including very high humidity (>80%) and high levels of

116   environmental dust within farming units (Kilpinen & Steenberg, 2009). Refinement of

117   materials selected for dusting could possibly have potential to extend the longevity of

118   this type of control, as could the use of dusts in liquid form. Schulz *et al* (2014),

119 however, reported no overall significant difference between liquid and dust form

120 silica-based controls.

121 There are prospects to develop novel control methods for PRM based on the use of

122 entomopathogenic fungi. Fungi produce extracellular chitinases which when

123 introduced to PRM chitin-rich hydrophobic coats can kill mites via desiccation (St *et*

124 *al.*, 1996). Fungi exhibit delayed pathology within PRM allowing for its wide

125 dissemination, thus eliminating large mite populations (Tavassoli *et al.*, 2008;

126 Tavassoli *et al.*, 2011). *Beauveria bassiana* has proved to be effective against PRM

127 more than 10 days post exposure (Steenberg & Kilpinen, 2003) whilst *Trichoderma*

128 *album* (Kaoud, 2010) and *Metarhizium anisopliae* fungi (Tavassoli, et al., 2011) are

129 efficient at high spore concentrations as new acaricides. The use of parasitic fungi as

130 a way to control PRM infestation could however generate downstream environmental

131 disequilibrium, since entomopathogenic fungi are generally not specific for PRM and

132 may affect other naturally existing insect populations.

133 Heat treatment is also regularly used to reduce PRM populations in egg laying units

134 in Norway and The Netherlands (M. Mul *et al.*, 2009). Heating hen houses to a

135   recommended 55°C kills PRM though it is suggested that high mite mortality also

136   occurs at 35°C (Tucci *et al.*, 2008). Heat treatment between flocks is not

137   recommended for controlling PRM by itself but as part of an integrated approach (M.

138   F. Mul & Koenraadt, 2009).

3. Digestive tract

140   The mite digestive tract is a comparatively well studied part of the anatomy of

141   several species including the storage mite *Lepidoglyphus destructor* (Erban &

142   Hubert, 2011), the house dust mite *Dermatophagoides farina* (Dumez *et al.*, 2014),

143   the sheep scab mite *Psoroptes ovis* (Hamilton *et al.*, 2003) and a range of

144   synanthropic species (Erban & Hubert, 2010). In combination these studies provide

145   an outline of the general anatomy of mites (Mehlhorn, 2001), although the specific

146   physiology of PRM, which are haematophagous mites, may be substantially

147   different.

148   It is largely accepted that the 'general' mite digestive tract is organised into three

149   recognisable parts; the foregut, midgut and hindgut. The foregut comprises the

150   pharynx and oesophagus extending posteriorly from the gnathosoma to the midgut.

151   Active food movement occurs through the oesophagus of PRM (J. Pritchard,

152   personal observation) presumably via the action of pharyngeal dilator muscles and

153   valves as has been demonstrated for *P. ovis* (Mathieson & Lehane, 2002).

154   The midgut, or ventriculus, and its associated caecae are thought to be primarily

155   responsible for PRM digestion as is for other haematophagous mites. The midgut is

156   located proximally between the third leg pairing and dorsally to most other internal

157   soft tissue including the malpighian tubules (see figure 2a+b). In unfed mites, the

158   midgut appears reduced in size but in engorged mites it expands to fill most of the

159   body cavity (Evans, 1992; Nisbet & Billingsley, 2000) as would be expected of a

160   haematophagous parasite that ingests large blood meals. Enlargement of the midgut

161   creates an increased surface area for digestive processes and also reduces the

162   distance of the midgut and caecae from internal organs that depend on nutrient

163   transport from the gut.

164   Acari midgut digestive cells are generally classified into three types (anterior midgut

165   cells, caecal cells and posterior midgut/hindgut cells) based on their function and

166   location. Anterior midgut epithelial cells contain large vacuoles and go through a

167   state of cytoplasmic degeneration whilst digesting food (Brody *et al.*, 1972; Coons,

168   1978). In engorged mites, these cells detach from the gut mucosa and are able to

169   engulf ingested material within the gut lumen becoming swollen and highly

170   vacuolated. The presence of intracellular large vacuoles that contain material of a

171   similar density to that seen in the gut lumen suggests that food digestion is carried

172   out at least in part intracellularly (Mathieson & Lehane, 2002). The autophagic-

173   lysosomal pathway is the most likely way that intracellular digestion occurs and is

174   thought to be initiated by the action of parasite endopeptidases such as Cathepsin D

175   and Cathepsin L (Nisbet & Billingsley, 2000). Vaccination of poultry with recombinant

176   PRM Cathepsin D or Cathepsin L induces anti-Cat D or anti-Cat L specific IgY

177   immunoglobulins and when these are ingested by PRM in an *in vitro* feeding system,

178   they cause increases in mite mortality (Bartley *et al.*, 2012). Most likely these IgY

179   antibodies bind directly to secreted Cathepsins D and L in the lumen of the mite gut

180   however vaccine-induced immunity is believed also to cause damage to the gut

181   barrier through direct binding of immunoglobins to membrane-bound proteins, even

182   though complement induced antibody upregulation may be required (Kemp *et al.*,

183   1989; Bartley, *et al.*, 2012).

184 PRM have six caecae extruding distally in a lateral manner, four anterior and two

185 posterior, all connected to the midgut in parallel to the third leg pairing (see figure

186 2a+b). Caecal epithelial cells in various mite species are densely packed with

187 lysosomes, smooth endoplasmic reticulum and mitochondria, all indicative of high

188 metabolic activity related to digestive enzyme activity. Brody *et al* (1972) proposed

189 that the lack of visible particulate material in the caecae of the house dust mite *D.*

190 *farinae* indicates that caecal cells secrete enzymes which are used for digestion in

191 the anterior midgut. However Erban and Hubbert (2011) demonstrated that midgut

192 and caecal-wide hydrolysis of fluorescent substrates by several proteolytic enzymes

193 occurred in the storage mite *L. destructor*. Given the significant expansion in size

194 and large volume of blood found in the caecae in engorged PRM (see figure 2a+b)

195 we suggest its caecae are also actively involved in food digestion.

196 The start of the hindgut in PRM is defined by the junction of two large malpighian

197 tubules at the posterior end of the midgut (see figure 2). Posterior midgut cells and

198 hindgut cells in several species of mite have been shown to be apically-basally

199 elongated with large microvilli (Brody, *et al.*, 1972; Mothes-Wagner, 1985). It is

200   believed the hindgut in mites is involved in water reabsorption and nutrient uptake,

201   though the mechanism is yet unclear. Water reabsorption creates a black food bolus

202   in PRM (J. Pritchard, personal observation) as seen also in *D. farinae* (Brody, *et al.*,

203   1972). Berridge and Gupta (1967) hypothesised that active transport of ions from the

204   rectal papillae of the blow fly into intercellular spaces causes an osmotic gradient

205   and thus water moves from the lumen to the hemolymph through osmosis. Further

206   understanding of water reabsorption in PRM could help identifying potential targets

207   for control.

208   The peritrophic membrane is another potential future target for control; its presence

209   has, however, neither been confirmed nor rejected in PRM. The presence of a

210   peritrophic membrane in some mites is well defined such as in the flour mite *Acaris*

211   *siro* (Hughes, 1950; Sobotnik *et al.*, 2008) but seemingly absent in others (Coons,

212   1978). The peritrophic membrane is a lamellar structure of chitin and associated

213   structural proteins, which surrounds the food bolus protecting the gut against

214   pathogenic microorganisms and compartmentalising food for digestive activity.

215   Sobotnik *et al.* (2008) reported that the ingestion of calcofluor (which binds chitin in

216   the membrane) and diflubenzuron (inhibits chitin synthesis) reduces *Acaris siro*

217   population growth. Interfering with chitin or the chitin associated proteins could be a

218   viable and safe method for PRM control since these molecules are absent in birds

219   and mammals. In haematophagous arthropods peritrophic membranes have been

220   suggested to protect epithelial cells against sharp edged haemoglobin crystals that

221   form with blood meals (Berner *et al.*, 1983; Eisemann & Binnington, 1994). In several

222   species of ticks the membrane has been described in great detail (Matsuo *et al.*,

223   2003; Zhu *et al.*, 1991) however as Eisemann & Binnington (1994) have noted,

224   targeting the peritrophic membrane in arthropods presents immediate difficulties.

225   This includes the possible destruction of antibodies and effector molecules from

226   vaccinated hosts within the proteolytic environment of the gut as well as the

227   necessity of a repeated control action every time a new peritrophic membrane is

228   formed during a new blood meal.

229   Proteins associated with the PRM midgut are not normally exposed to the avian

230   immune system during mite feeding so the bird host does not generate a natural

231   antibody response to them. These 'concealed' gut antigens within the PRM

232 therefore have potential to be selected as targets for vaccination as antibodies from

233 vaccinated bird hosts would be taken up in a mite blood meal. Immunising hosts with

234 gut-derived concealed antigens has proven successful for development of the

235 vaccine TickGARD® (Hoechst Animal Health; Australia) against the midgut-

236 expressed BM86 protein of the cattle tick *Rhipicephalus microplus* (Willadsen *et al.*,

237 1995). Though no homolog to BM86 has been found in PRM the same strategy has

238 recently been pursued using other internally expressed proteins (Arkle *et al.*, 2008;

239 Bartley *et al.*, 2009; Bartley, *et al.*, 2012).

240 ## 4. Nervous system

241 Acari, including PRM, have a clustered region of nervous tissue known as the

242 synganglion in the anterior section of the idiosoma, just anterior to the midgut (see

243 figure 3). In PRM this central nervous mass is separated into two regions, the supra-

244 oesophageal nervous mass and the sub-oesophageal nervous mass. In agreement

245 with Serverino *et al* (1984) we describe four pairs of pedal ganglia extending distally

246 from the supra-oesophageal mass (Figure 3a), each ganglion connecting to each of

247   the eight legs of the mite. The sub-oesophageal mass (figure 3b) is bisected

248   longitudinally by the oesophagus and surrounded by fat tissue.

249   Chemical acaricides against PRM predominantly target neurotransmitters and

250   synapses between neurons within the synganglion tissue (see figure 4). These

251   substances classically target the voltage-gated Na^+ channels of pre-synaptic axons,

252   propagating a continually depolarised membrane leading to loss of action potential

253   and eventually mite paralysis. Mites that cannot move to find food or escape

254   environmental factors eventually die. Sprayed acaricides are most likely taken up

255   via sites of gaseous exchange in the PRM principally through the stigmata, located

256   adjacent and dorsally to coxae II and III, through the peritreme branching network,

257   into the haemolymph and finally through to the synganglion tissue. Mite synganglion

258   tissue is reported in several mite species to be covered in an acellular sheath of

259   neural lamellae which allows access of nutrients and other compounds (Coons &

260   Axtell, 1971; Woodring & Galbraith, 1976). A rind of perikaryon (neural somata) cells

261   further surrounds a central neuropile of axons and dendrites (Severino, *et al.*, 1984)

262   where it is likely PRM neurological controls are mostly active.

263   PRM populations are known to be resistant to earlier generations of neurological

264   pesticides, such as dichlorodiphenyltrichloroethane (DDT) and the pyrethroids

265   (Zeman & Zelezny, 1985; Beugnet, *et al.*, 1997). DDT is now banned for pesticidal

266   control within the EU (UNEP-Chemicals, 2006) as it accumulates to high

267   concentrations in food chains, persists in the fatty tissues of animals and humans,

268   and is associated with risk of several chronic illnesses (Orris *et al.*, 2000; Eskenazi *et*

269   *al.*, 2006).   Pyrethroids are no longer used extensively with the exception of

270   permethrin, a 3rd generation synthetic compound with activity against insects and

271   acari (Blagburn & Dryden, 2009). Pyrethroid resistance has been reported in the

272   important mite species *Varroa destructor* (Unit, 2013) and *Sarcoptes scabiei*

273   (Andriantsoanirina *et al.*, 2014). The use of pyrethroids has also been associated

274   with increased numbers of *Tetranychus urticae* due to its toxicity against predatory

275   mites of this species (Penman & Chapman, 1988).

276   Other commercially popular pesticides include organophosphates such as Phoxim

277   (Bayer, Germany), which target acetylcholinesterse, a hydrolytic enzyme required for

278   acetylcholine hydrolysis and cross-synaptic signal termination. Acetylcholine is

279   essential for neuron-to-neuron excitatory signal transmission thus inhibition of signal

280   termination by Phoxim overloads receptors with too much acetylcholine preventing

281   recovery of post-synaptic neuron potential. 50% Phoxim (Byemite®, Bayer,

282   Germany) shows acaricidal effect on all stages of PRM as well as on egg

283   development (Meyer-Kühling *et al.*, 2007), although resistance may have already

284   arisen in some natural populations in Poland (Zdybel *et al.*, 2011). Post-synaptic

285   acetylcholine receptors are also targeted by naturally derived essential oils and

286   spinosyn A via competitive inhibition. Conversely, these compounds hinder

287   acetylcholine binding so no post-synaptic signal is produced. Spinosad acaricides

288   are a mixture of the compounds spinosyn A and D. Unlike spinosyn A which binds

289   post synaptic acetylcholine receptors, spinosyn D targets GABA (gamma-

290   aminobutyric acid) receptors (Orr *et al.*, 2009). Focusing acaricidal controls on two

291   different target receptors of acetylcholine and GABA reduces the chance of natural

292   resistance of mite populations to spinosad controls. The neurotransmitter GABA

293   acts, in contrast to acetylcholine, by inhibiting excitatory signals. This suppression is

294   enhanced by abamectin/ivermectin controls which stimulate GABA release in pre-

295   synaptic neurons and enhance its post-synaptic binding to GABA receptors. This

296   induces hyperpolarisation of post-synaptic membranes via increased flow of chloride

297   ions thus affecting downstream signalling capabilities.

298   Due to the conserved nature of acari and insect neural pathways several acaricides

299   are effective against many co-inhabiting species. The use of such substances, albeit

300   practical, increases the risk of ecological disequilibrium. In addition the concurrent

301   use of controls that target similar pathways increases the likelihood of resistance

302   selection to multiple controls as has been seen in other insects and arthropods

303   (Acevedo *et al.*, 2009; Fernández-Salas *et al.*, 2012)

5. Salivary gland proteins

305   Salivary gland proteins in haematophagous arthropods, including many acari

306   species, have been shown to have biological functions in blood feeding. These

307   proteins can influence blood flow through antihemostatic properties (Champagne,

308   2004), interact with host immune cells to cause immunomodulation (Schoeler &

309   Wikel, 2001; Titus *et al.*, 2006) and eliminate bacteria in the feed by displaying anti-

310   microbial properties. Salivary proteins of the cattle tick *Rhipicephalus annulatus* have

311   been suggested as potential alternative vaccine candidates (Shahein *et al.*, 2013) as

312   there is concern that 'concealed' or 'hidden' antigens from tick guts such as the

313   BM86 vaccine TickGARD may not be effective in species other than *R. microplus*

314   (Willadsen, *et al.*, 1995; Nuttall *et al.*, 2006). Unlike mites, ticks generally feed on

315   their hosts for days or weeks at a time. This prolonged period of feeding requires the

316   production of bioactive lipids and proteins in the salivary glands which are used to

317   cement the tick to the biting site as well as to fight host immune-regulation,

318   haemostasis and inflammation (Steen *et al.*, 2006; Francischetti *et al.*, 2009). It is

319   possible that PRM salivary gland proteins are taxonomically related to known tick

320   salivary gland proteins, however PRM feeding time is much shorter. A recent

321   publication on sequencing the PRM transcriptome identified 24 potential salivary

322   proteins likely to be involved in blood digestion (Schicht *et al.*, 2013) some of which

323   have hypothesised anti-bacterial functions.

324   Secreted proteins in the saliva of the honey bee mite *V destructor* damage insect

325   haemocytes and prevent aggregation formation that occurs in host wound healing

326   (Richards *et al.*, 2011). The requirement of *V. destructor* populations to feed multiple

327   times on the same host is reflected in PRM behaviour, although it is unclear whether

328   PRM feed repeatedly on the same open wound similarly to. If this is the case anti-

329   healing proteins may be a viable control target when present in PRM. More likely

330   targets however are secreted salivary proteins with anti-microbial function since

331   pathogens are ingested with blood meals regardless of feeding time duration.

332   Studies into anti-microbial salivary proteins in ticks (Yu *et al.*, 2006; Liu *et al.*, 2010)

333   as well as other arthropods (Titus, *et al.*, 2006) should benefit further PRM research.

6. Alternative and novel targets

Mechanical and sensory inhibition

Mites do not have eyes but sense their environment through hair-like appendages

called setae, normally clustered at the palpal or tarsal extremities. In general, setae

sense vibration, heat, moisture, CO_2 or chemical cues generated by hosts or

potential mates. PRM setae in the forelegs and palps play important roles in both

olfactory and mechanical sensing (Cruz *et al.*, 2005) as evidenced by increased

movement of PRM in response to small vibrations and increases in environmental

heat, suggestive of the presence of a passing host (Kilpinen, 2001). Kilpinen (2001)

demonstrated that PRM exhibit increased heat-induced movement 2-10 days post-

feeding compared to mites fed 1 day before or fed >10 days before. Interestingly this

correlates to the physiology of blood digestion in PRM suggesting that hungry mites

2-10 days post feeding exert more energy on host finding, but after 10 days they

become more static to conserve energy. PRM undergo a stasis-like diapause if no

host is present or if the temperature drops, which is reflected by seasonal variations

reported in PRM numbers (Nordenfors & Hoglund, 2000).

350   The potential of utilising CO_2, olfaction, and micro-vibrations in control strategies

351   discussed below.

Disrupting mating behaviour using micro-vibrations

353   Both females and males of various species of insects produce and react to micro-

354   vibrations thought to be involved with mate attraction. Predominantly this behaviour

355   has been studied in tree and plant parasitising species including the American

356   grapevine leafhopper *Scaphoideus titanus* (Mazzoni *et al.*, 2009), the southern green

357   stink bug *Nezara viridula* (de Groot *et al.*, 2010), the Asian citrus psyllid, *Diaphorina*

358   *citri* (Rohde *et al.*, 2013) and the southern pine beetle *Dendroctonus frontalis* (Aflitto

359   & Hofstetter, 2014). Studies have shown conspecific vibration patterns such as those

360   from competing males (Mazzoni, *et al.*, 2009; Rohde, *et al.*, 2013) or heterospecific

361   patterns such as those from a predator (de Groot, *et al.*, 2010), can alter male

362   behaviour resulting in reduced mating events.

363   PRM are a colony-developing species and therefore mating may simply be a random

364   process or pheromone-dependent (Entrekin & Oliver, 1982; Koenraadt & Dicke,

365   2010), rather than directed by vibration. Mite activity is increased when PRM are

366   exposed to substrate-borne microvibrations at 2 kHz (Kilpinen, 2005) however this

367   has not been suggested to be directly related to mating behaviour. Further work into

368   PRM reproductive behaviour and vibration sensing is needed to understand if this

369   could be a potential route for population control.

370   **Use of carbon dioxide / mite traps**

371   *D. gallinae* initially remain static in the presence of CO_2 although after 2 minutes

372   exposure they display higher rates of movement compared to those of unexposed

373   PRM (Kilpinen, 2005). This correlates to the behaviour of other haematophagous

374   arthropods such as mosquitoes and ticks where CO_2 induces increased movement

375   based on evolution of host seeking behaviour. CO_2 producing traps can be used as

376   attractant controls as demonstrated by Garcia and others (Garcia, 1962; Newhouse

377   *et al.*, 1966; Wilson *et al.*, 1972). Carbon dioxide has also been considered for

378   control of several species of phytophagous mites that feed on stored crops (White &

379   Jayas, 1991; Conyers & Bell, 2003). Using levels of 50-60% CO_2 in enclosed

380   storage units reduces mite numbers significantly by asphyxiation however the use of

381   CO_2 at these levels is not appropriate for PRM control in farming units housing

382   poultry flocks. The use of local CO_2 gradients to attract PRM into the vicinity of an

383   already established PRM trap could be a potential alternative approach. Cardboard

384   traps coated in compounds with acaricidal properties have proved to be a simple but

385   effective control measure in trials in Sweden (Chirico & Tauson, 2002).

386   Implementation of CO_2 producing products for large scale control does remain

387   speculative given the dangerously high levels that would be required for larger

388   farming units. More appropriate would be their implementation in an integrated

389   approach using multiple control methods.

390   ## Predators and olfactory perception

391   Olfactory receptors in PRM are suggested to play a role in mite survival since PRM

392   remains initially and transiently motionless upon sudden CO_2 concentration increase

393   (Kilpinen, 2005). The CO_2 increase possibly mimics the presence of potential

394   predators. Consistently higher levels of CO_2, however, induce PRM movement,

395   suggesting perhaps a situation when their immediate risk of danger ceases to exist.

396   PRM colonies that are openly exposed to hen flocks in illuminated areas are quickly

397   pecked and presumably eaten (J. Pritchard, personal observation) thus explaining

398   why PRM usually inhabit dark enclosed spaces and are nocturnal feeders. Use of

399   intermittent light regimes has shown to vary mite numbers captured in studies carried

400   out in Poland (Sokół *et al.*, 2008) however application of lighting regimes in poultry

401   houses varies between countries and such maybe subject to poultry welfare laws.

402   Several predatory species including *Hypoaspis miles*, *Hypoaspis aculeifer*,

403   *Amblyseius degenerans* and *Phytoseiulus persimilis* are able to feed on *D. gallinae*,

404   though feeding success as part of experimental PRM controls have proven to be

405   dependent on environmental conditions and absence of alternative prey (Lesna *et*

406   *al.*, 2009; Ali *et al.*, 2012; Lesna *et al.*, 2012). The predatory mite *P. persimilis* feeds

407   predominantly on the spider mite *Tetranychus urticae* and has been shown to be

408   attracted to volatile compounds produced by plants fed on by *T. urticae* (Drukker *et*

409   *al.*, 2000; De Boer & Dicke, 2004). A hypothetical PRM control could be, for instance,

410   the addition of such predator attractants to areas typically inhabited by PRM.

411   *D. gallinae* themselves are affected by volatile compounds, most notably   repellent

412   substances (Soon-Il *et al.*, 2004; George, Olatunji*, et al.*, 2010; George, Sparagano*,*

413   *et al.*, 2010). Plant derived essential oils are shown to possess repellent and even

414   lethal characteristics of which garlic and thyme oils appear to be the most effective.

415   As reviewed by George *et al.* (2014) naturally derived essential oils benefit from low

416   mammalian toxicity and short environmental persistence indicating their potential

417   future use as part of integrated control strategies.

418   Conversely, little research has been carried out into mite attracting compounds.

419   Zeman (1988) showed attraction of PRM to host-derived bird surface skin lipids

420   which is postulated to be part of the evolution of PRM host-detection. Furthermore *D.*

421   *gallinae* have been shown to release pheromones which attract other PRM causing

422   mites to cluster together, most likely for protection (Entrekin & Oliver, 1982;

423   Koenraadt & Dicke, 2010). How repellent or attractant compounds are used in future

424   controls would require further research. The study of attractants to be employed in

425   mite traps, repellents to be employed in densely populated areas and mechanical

426   constraints would be beneficial for the development of integrated control strategies.

Embryogenesis

428   Adult female PRM are oviparous, laying 3-4 eggs after mating. Oviposition time

429   varies with temperature but is suggested to be on average 1-3 days at 20-45°C

430   (Maurer & Baumgartner, 1992; H. Nordenfors, *et al.*, 1999). Embryo development

431   requires various compounds including proteins, sugars and lipids which are secreted

432   from both ovarian and extra-ovarian tissues. These compounds include vitellogenin,

433   the precursor for the yolk protein vitellin, an essential nutrient during early

434   embryogenesis (Seixas *et al.*, 2012). A range of proteases involved with the

435   hydrolysis of vitellin, leading to yolk degradation, have been isolated in eggs of the

436   cattle tick *R. microplus* (Logullo *et al.*, 1998; Sorgine *et al.*, 2000; Seixas *et al.*, 2008)

437   and targeted via vaccination. This has led to reduction in tick fecundity and next

438   generation egg weight in ticks fed on the blood of vaccinated bovine hosts (da Silva

439   Vaz *et al.*, 1998; Seixas, *et al.*, 2008). Of these proteases vitellin-degrading cysteine

440   endopeptidase (VTDCE), a Cathepsin-L like protein, is the most active enzyme.

441   Comparative study into embryogenesis in PRM is lacking, but homologues to

442   Cathepsin-L have been identified through suppression subtractive hybridization

443   (Bartley, *et al.*, 2012). Wright *et al* (2011) identified vitellogenin in PRM as the protein

444   with the highest difference in expression between cDNA libraries of fed and unfed

445   mites. Due to the increase in expression Cat-L and vitellogenin in fed mites it is

446   plausible that Cat-L like proteases could play a part in PRM vitellogenesis. Huntley *et*

447   *al* (2004) describe a vitellogenin homologue in the sheep scabies mite *P. ovis* to be

448   highly immunogenic to the host. It is hypothesised *P. ovis* may induce allergic

449   response to aid feeding and thus pre-vaccination of allergens such as vitellogenin

450   may inhibit the induction of pro-inflammatory IgE antibodies and influence mite

451   feeding. Success of PRM control is often measured at population level through total

452   mite numbers, egg counts, analysis of rates of oviposition and development of early

453   stage PRM. Embryogenesis and its associated molecules such as vitellin are

454   therefore suggested as attractive potential future control targets.

455   ## The Haemocoel / Immune system

456   Jasinskas *et al* (2000) reported the ability of immunoglobulins specific to a range of

457   tick proteins to cross from a blood meal to the haemolymph of the lone star tick

458   *Amblyomma americanum* through the midgut epithelium. This proof of concept in

459   ticks suggests there is a possibility of raising antibodies against essential proteins for

460   ticks/mites present in the hemolymph and fat body. The acari immune system is

461   composed of phagocytising haemocytes and anti-microbial peptides such as

462   defensins and lysozymes. The midgut is the primary site for destruction of bacterial

463   and viral pathogens which are ingested with a blood meal, but if these microbes

464   successfully traverse the midgut epithelium, then defensins and lysozymes are

465   secreted into the haemolymph and fat body (Ceraul *et al.*, 2003; Simser *et al.*, 2004;

466   Taylor, 2006). Lysozymes in astigmatid mites can function in both defence and also

467   in digestion when microbes are used as a secondary food source (Childs & Bowman,

468   1981; Erban & Hubert, 2008). Greater understanding of PRM lysozymes and the

469   cells that contain them could contribute to novel controls against the mites by

470   affecting the ability of the mite to process ingested pathogens that may affect or be

471   transmitted by PRM, as demonstrated for ticks (Simser, *et al.*, 2004).

472   Infection of PRM with bacteria has been shown by Valiente Moro *et al* (2009) who

473   demonstrated that *Salmonella enteritidis* can enter the PRM

474   heamolymph/reproductive organs and infect protonymphs via transovarial passage.

475   Valiente Moro *et al* further demonstrated the negative effect of bacterial infection on

476   PRM fecundity, with only 31% oviposition in infected PRM compared to 68%

477   oviposition in control PRM. This suggests that targeting the PRM immune system

478   and thus affecting their ability to cope with pathogens such as *S. enteritidis* in the

479   reproductive organs could be explored.

480   Subolesin, a tick homologue of the mammalian akarin family of proteins, is

481   associated with the upregulation of innate immunity in various tick species (Zivkovic

482 *et al.*, 2010) and is proposed to be a transcription factor involved in multiple cellular

483 processes (De la Fuente *et al.*, 2008). Harrington *et al* (2009) showed that

484 immunisation of chickens with recombinant *Aedes albopictus* subolesin increased

485 fed PRM mortality by 31% compared to control groups, suggesting that a potential

486 PRM subolesin orthologue may be a target for control. RNA interference of the

487 subolesin gene in ticks has shown varying efficacy in terms of how well ticks are able

488 to control bacterial infections. Zivkovic *et al* (2010) demonstrated that RNAi knock-

489 down of subolesin in ticks increased infection by *Francisella tularensis* but decreased

490 infection by *Anaplasma marginale*. Whether by means of immunological repression

491 resulting in increased bacteria loads or affecting other PRM systems, subolesin

492 would make an interesting target for further vaccine studies against PRM.

7. Integrated Control Strategies

494 The efficiency of PRM control is dependent on many factors including substances

495 employed, farm layout, mite population numbers and environmental factors. Future

496 improvements to PRM control therefore will likely require integrated strategies such

497 as the Hazard Analysis and Critical Control Points (HACCP) method laid out by Mul

498   and Koenraadt (2009). The efficacy and longevity of new control strategies, such as

499   the introduction of vaccines or novel acaricides, are likely to be affected by specific

500   farming practices and methods of animal husbandry (Harrington *et al.*, 2011) and will

501   require careful planning. For example, introduction of novel acaricides to a system

502   using natural predators of PRM may also affect the predator species as well as *D.*

503   *gallinae* (Harrington *et al.*, 2011).

8. Concluding remarks

505   The variable nature of control strategies taken by each farmer, ongoing changes in

506   caged poultry regulations and the rapid emergence of acaricidal resistance, suggests

507   that PRM will continue to be a major problem to the global egg-laying industry.

508   Understanding PRM biology is essential for developing improvements to current

509   biological controls and should be at the forefront of any future PRM research. In this

510   short review we have identified several biological targets that offer potential for

511   possible future controls against PRM including embryogenesis, food digestion,

512   sensory perception and predatory intervention. The current lack of a single

513   commercial control methodology means that research into these fields would be of

514   enormous benefit to the poultry industry and commercial sector.

515

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10. References

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861 **Figure 1: The life cycle of** *Dermanyssus gallinae*. The life cycle of PRM can be

862 completed in 7 days, from egg to adult (Maurer & Baumgartner, 1992) although 14

863 days is more usual. Commonly only females of the protonymph, deutonymph and

864 adult stages feed on blood, though males have been known to feed. Female adults

865 typically lay clutches of 4-8 eggs with a maximum of 30 eggs total in their life time.

866 Larvae have 6 legs (not 8 as the other stages) and all stages live off the host,

867 feeding intermittently for short periods at a time.

868 **Figure 2 Comparison of the PRM digestive system in blood fed (2a) and unfed (2b)**

869 **mites.** Mites were observed at x100 magnification from the dorsal side. Gnth –

870 Gnathosoma (mouthparts), Os – Oesophagus, Ca I-III – Caeca I-III, Mp – Malpighian

871 tubules, Hg – Hindgut. The PRM digestive tract extends from the gnathosoma

872 posteriorly through the oesophagus, midgut and caeca and ending in the hindgut.

873 Most blood digestion occurs in the much expanded three caecal pairings (Ca I-III)

874 and central midgut (Mg) (Figure 2a). Malpighian tubules elongate longitudinally

875 along the idiosoma connected to the anterior hindgut (Figure 2b). These are involved

876 in nitrogenous waste collection and osmoregulation. Waste leaves through the

877 posterior hindgut and through the anal opening (not shown). Note: mite body shape

878 increases and gets rounder during feeding and the digestive tract completes most of

879 the body cavity of the PRM when full (Figure 2a) compared to that of an unfed mite

880 (figure 2b).

881 **Figure 3: The synganglion tissue (brain) of the PRM.** Longitudinal sections of 10μm

882 thickness observed at x200 magnification. Sections were stained with 1:100 anti-

883 Cathepsin-D chicken IgY (kindly donated by Dr Alisdair Nisbet) then 1:1000 goat

884 anti-rabbit IgG HRP and counter stained with haematoxylin. Pg I-IV – Pedal ganglion

885 1 to 4, SpCNM – Supra-oesophageal central nervous mass, Sb – Sub-oesophageal

886 mass, Es –Oesophagus. The PRM synganglion tissue, as in all acari, is divided by

887 the oesophagus into two connected masses – the supra-oesophageal mass (Figure

888 3a) and the sub-oesophageal mass (Figure 3b). Figure 3a shows the supra-

889 oesophageal central nervous mass connected to 8 pedal ganglia extending distally

890 to each corresponding leg. Figure 3b shows the sub-oesophageal mass,

891 comparatively more rounded, split by the oesophagus extending longitudinally down

892 though the centre.

893 **Figure 4: Neurological targets for acaricidal controls against *D. gallinae*.** Pesticides

894 and other controls affect either the transmission of acetylcholine (secreted from an

895 excitatory neuron shown in red) required for excitatory signals or gamma-

896 aminobutyric acid (GABA) (secreted from an inhibitory neuron shown in blue) which

897 are the predominant inhibitory neurotransmitters in the nervous system. Competitive

898 inhibition of acetylcholine and GABA through binding to post-synaptic receptors is a

899 common mode of action for acaricides. An alternative mode of action is the binding

900 and inactivation of the enzyme acetylcholinesterse, which is required to hydrolyse

901 acetylcholine and end signalling, thus leading to overstimulation. Several pesticides

902 bind to and over stimulate the voltage gates Na^+ channels in the presynaptic axon.

903 These mechanisms aim to induce paralysis and consequently lead to death in red

904 mite through excitoxicity and overstimulation in neural pathways or conversely

905 through transmission inhibition.