

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

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1 Understanding the Biology & Control of the Poultry Red Mite,

2 *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).

139 **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

therefore have potential to be selected as targets for vaccination as antibodies from vaccinated bird hosts would be taken up in a mite blood meal. Immunising hosts with gut-derived concealed antigens has proven successful for development of the vaccine TickGARD® (Hoechst Animal Health; Australia) against the midgut-expressed BM86 protein of the cattle tick *Rhipicephalus microplus* (Willadsen *et al.*, 1995). Though no homolog to BM86 has been found in PRM the same strategy has recently been pursued using other internally expressed proteins (Arkle *et al.*, 2008; Bartley *et al.*, 2009; Bartley, *et al.*, 2012).

4. Nervous system

Acari, including PRM, have a clustered region of nervous tissue known as the synganglion in the anterior section of the idiosoma, just anterior to the midgut (see figure 3). In PRM this central nervous mass is separated into two regions, the supra-oesophageal nervous mass and the sub-oesophageal nervous mass. In agreement with Serverino *et al.* (1984) we describe four pairs of pedal ganglia extending distally 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 ring 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 acetylcholinesterase, 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)

304 **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, CO₂ 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 CO₂, olfaction, and micro-vibrations in control strategies
351 discussed below.

352 **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; Koenraad & Dicke,
365 2010), rather than directed by vibration. Mite activity is increased when PRM are

exposed to substrate-borne microvibrations at 2 kHz (Kilpinen, 2005) however this has not been suggested to be directly related to mating behaviour. Further work into PRM reproductive behaviour and vibration sensing is needed to understand if this could be a potential route for population control.

Use of carbon dioxide / mite traps

D. gallinae initially remain static in the presence of CO₂ although after 2 minutes exposure they display higher rates of movement compared to those of unexposed PRM (Kilpinen, 2005). This correlates to the behaviour of other haematophagous arthropods such as mosquitoes and ticks where CO₂ induces increased movement based on evolution of host seeking behaviour. CO₂ producing traps can be used as attractant controls as demonstrated by Garcia and others (Garcia, 1962; Newhouse *et al.*, 1966; Wilson *et al.*, 1972). Carbon dioxide has also been considered for control of several species of phytophagous mites that feed on stored crops (White & Jayas, 1991; Conyers & Bell, 2003). Using levels of 50-60% CO₂ in enclosed storage units reduces mite numbers significantly by asphyxiation however the use of CO₂ at these levels is not appropriate for PRM control in farming units housing poultry flocks. The use of local CO₂ 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 CO₂ 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 CO₂ concentration increase
393 (Kilpinen, 2005). The CO₂ increase possibly mimics the presence of potential
394 predators. Consistently higher levels of CO₂, 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.

427 **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 haemolymph/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 akarín family of proteins, is
481 associated with the upregulation of innate immunity in various tick species (Zivkovic

et al., 2010) and is proposed to be a transcription factor involved in multiple cellular processes (De la Fuente *et al.*, 2008). Harrington *et al* (2009) showed that immunisation of chickens with recombinant *Aedes albopictus* subolesin increased PRM mortality by 31% compared to control groups, suggesting that a potential PRM subolesin orthologue may be a target for control. RNA interference of the subolesin gene in ticks has shown varying efficacy in terms of how well ticks are able to control bacterial infections. Zivkovic *et al* (2010) demonstrated that RNAi knock-down of subolesin in ticks increased infection by *Francisella tularensis* but decreased infection by *Anaplasma marginale*. Whether by means of immunological repression resulting in increased bacteria loads or affecting other PRM systems, subolesin would make an interesting target for further vaccine studies against PRM.

7. Integrated Control Strategies

The efficiency of PRM control is dependent on many factors including substances employed, farm layout, mite population numbers and environmental factors. Future improvements to PRM control therefore will likely require integrated strategies such 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).

504 **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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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 acetylcholinesterase, 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 excitotoxicity and overstimulation in neural pathways or conversely
905 through transmission inhibition.