

## Review article

# Biology and genetics of the poultry red mite (*Dermanyssus gallinae*) – new targets for eradicating and controlling invasion

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**ABSTRACT.** *Dermanyssus gallinae* is considered to be one of the world's most troublesome ectoparasites of laying hens, with the consequences for the hens being reflected in production rates, including reduced egg quantity and quality. Despite intensive efforts to develop research on this mite, it remains challenging to combat. This article reviews current and recent knowledge on the biology and genetics of *D. gallinae*, and new targets for control are discussed as identified by the authors of recent publications. It is imperative to acknowledge the significance of this knowledge in order to facilitate the identification of genetic markers associated with the emergence of acaricide resistance. This, in turn, will enable the development of alternative strategies and methods to control *D. gallinae*.

Keywords: PRM, laying hen, ectoparasite, control, combat strategy, control target

## Introduction

*Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanissidae) also known as poultry red mite (PRM) is considered one of the most troublesome ectoparasites in industrial laying hen production. It has been found in many countries around the world in different types of housing, regardless of the destination and size of the flock [1]. The population of the mite grows rapidly under industrial farming conditions, making eradication a challenging task. This is partly due to the behaviour of the mite and the development of its resistance to acaricides. *D. gallinae* is not a permanent parasite. It is a temporary parasite that, after the blood meal (which lasts approximately 0.5–1.5 hours), exits the host and moves to the host site where it reproduces and undergoes its developmental cycle. Feeding occurs at intervals of 2–4 days, mainly during nocturnal periods when bird activity is reduced [2–5]. Microenvironmental factors that favour the proliferation and mass occurrence of the parasite include the crevice-rich structure of the poultry

house, the high bird density in a confined area of the poultry house, and the constant high temperature and humidity [1,4,5]. In cage systems, infestation intensity by *D. gallinae* can reach approximately 50,000 individuals per bird, and in severe cases up to 500,000 [1,6]. *Dermanyssus gallinae* causes a parasitic disease known as dermanyssosis, which can lead to a decline in bird welfare and condition, reduced laying rate and egg quality, and increased mortality [6–9,11]. The infestation has been shown to cause severe economic losses in the poultry sector, estimated at €130 million in 2004 [12] and €231 million in 2017 [13]. *Dermanyssus gallinae* remains a challenge.

The aim of this article is to provide an overview of recent reports on the biology and genetics of *D. gallinae*, in which scientists are typifying new targets in the fight against the mite. This knowledge is crucial for identifying genetic markers of emerging acaricide resistance and developing alternative strategies and methods to control the mite [14–16].

## Biology of *D. gallinae*

A description of the biology of *D. gallinae* with reference to the source literature can be found in a previous paper [17]. Here, only selected topics are cited and enriched with recent data. *D. gallinae* is a small mite with a length of 0.7 to 1 mm and a width of 0.4 to 0.5 mm. The colouration of *D. gallinae* varies depending on the degree of blood digestion; it ranges from grey (complete digestion of blood) through red to brown. The body of *D. gallinae* is divided into two regions: the gnathosoma, comprising the mouth opening, chelicerae and pedipalp segments, and the idiosoma. The adult form is pear-shaped, dorsoventrally flattened and covered with a chitinous carapace. The cuticle is composed of various structures, including bristles, glands, and shields, which are categorised into the following regions: pectoral, genital, abdominal, and anal. The cuticle is further subdivided into the epi-, exo-, and endocuticle. The epicuticle is comprised of two layers: the inner layer, which consists of lipids, phenols, and proteins; and the outer layer, which consists of waxes. The primary function of the epicuticle is to act as a barrier against the penetration of pesticides [18]. The life cycle of the insect includes five stages: egg, larva, two nymphs (protonymph and deutonymph), and the adult form (male or female). The forms that engage in a parasitic lifestyle, characterised by blood-sucking, include the protonymphs, deutonymphs, and mature females. The adult stage is characterised by copulation following the final instar. Subsequent to this, the female will feed on blood and, after a period of three days, will lay a packet of eggs, the number of which depends on environmental conditions, but is usually about eight [2,19]. The duration of this stage is approximately one and a half to two days, and the larva subsequently undergoes a transformation into a protonymph. The transition from the protonymph stage to the deutonymph stage, and subsequently from the deutonymph stage to the adult form, necessitates the extraction of blood from the host on each occasion, occurring successively after approximately 24- and 48-hours [2].

The life cycle of *D. gallinae* is influenced by environmental conditions such as access to the host, ambient temperature and relative humidity (RH) with an average duration of 2 weeks. Under abiotic conditions maintained at a constant 20–25°C and RH>70%, the development cycle can be reduced to

7–10 days – the number of parasites can double within a week [7,20]. *Dermanyssus gallinae* is a species very resistant to adverse conditions. Without feeding, nymphs can survive for 8–9 months at 5°C and approximately 6 weeks at 25°C. Temperatures below –20°C (50% RH) and above 45°C (11%) are lethal [21].

## Reproduction and male *D. gallinae*

Research into the reproductive biology of *D. gallinae* under laboratory conditions is challenging due to the mite's tendency to disperse in open spaces and hide in crevices. However, recent advancements in techniques have enabled the closed culture of these parasites, thereby preventing their dispersal, and the development of an artificial feeding method that greatly facilitates observation. Oliver [22] demonstrated that female of *D. gallinae* require host blood to oviposit. Recent studies have demonstrated that fertilisation occurs exclusively in adult females, who are capable of laying eggs [23]. It has been determined that the maximum number of oviposition events in a lifetime for adult females is 13, with an average oviposition rate of approximately 44 eggs [23]. A male mite can mate with an average of 16 females during its lifetime, with the average mating period being 8.47 days [23]. Furthermore, it has been demonstrated that only males hatch from the first batch of eggs laid by new adult females, with the proportion of male offspring gradually decreasing with each generation [23]. This highlights the importance of males in the establishment of *D. gallinae* populations, suggesting that they can be considered a promising target for mite control [23].

## The process of vitellogenesis

Vitellogenesis in *D. gallinae*, the process of formation of spare material in the egg cell, has been the subject of recent research. It has been demonstrated that this process is induced by the ingestion of blood and is essential for reproduction of mite. The genes responsible for this process are called *Vg*. Bartley et al. [24] identified gene called *Vg1* and demonstrated its potential as a vaccine against *D. gallinae*. Ribeiro et al. [25] identified three *Vg* homologues in the transcriptome of adult *D. gallinae*. Liu et al [26] indicates the presence of a family of four homologous genes called *Dg-Vg1*, *Dg-Vg1-like*, *Dg-Vg2* and *Dg-Vg2-like*. Cited study

demonstrated the structure and function of the listed genes. Study showed expression levels of all *Vg* genes were significantly higher in adult females than in other developmental stages, with the expression of these genes increasing after the mites had taken blood and then decreasing [26]. Silencing of *Dg-Vgs* by RNA interference (RNAi) resulted in reduced female fecundity and egg hatching rates, and abnormalities in embryo development. Silencing of the *Dg-TOR* gene (*TOR* proteins belong to the family of phosphatidylinositol 3-kinase and are targets of the antiproliferative drug rapamycin) led to a substantial decrease in *Dg-Vgs* expression and exerted a detrimental effect on *D. gallinae* reproductive capacity, suggesting that *TOR* impacts *D. gallinae* reproduction by modulating *Dg-Vgs* expression [26]. The researchers underscore the pivotal role of *Dg-Vgs* and *Dg-TOR* genes in *D. gallinae* reproduction, underscoring their potential as a target for mite control.

### Body surface hydrophobicity

Researchers have identified the role of *D. gallinae* surface hydrophobicity as a significant factor contributing to the reduction in effectiveness of applied sprays. Studies have demonstrated that the surface of *D. gallinae* exhibits hydrophobic characteristics. The microstructures of the surface of *D. gallinae* have been observed to comprise cuticular folds, with a lipid-rich outer cuticular layer [27]. The primary components of the cuticular lipids are fatty acids and n-alkanes [27]. The chemical composition and microstructures of the surface have been demonstrated to play a pivotal role in determining the hydrophobicity of the surface, thereby hindering the penetration of compounds into the cuticle. The addition of surfactants to the pesticide solution has been shown to enhance its wettability, thereby overcoming surface hydrophobicity and increasing the effectiveness of the pesticide against mites [27].

### Gut microbiota

The role of the gut microbiota in organisms is of significant importance in maintaining homeostasis and also exerts a considerable influence on fitness in arthropods. The intestinal microbiota of *D. gallinae* was found to be dominated by bacteria from the genera *Bartonella* and *Kocuria* sp. Recent studies

demonstrate the involvement of the microbiota in the digestion of blood taken by the mites [28]. Cited study showed a significant increase in *Bartonella* A abundance was observed subsequent to the mites' ingestion of blood, along with the presence of haemolytic activity in some bacterial strains isolated from *D. gallinae* on blood agar. Experimental evidence demonstrated that mites subjected to a selective deprivation of microbiota (following antibiotic administration) exhibited delayed blood digestion and diminished reproductive capacity. Notably, the *Bartonella* species detected in *D. gallinae* represents a novel taxon [28]. This novel species, designated as *Bartolella* A, exhibits characteristics of an obligatory symbiont. The findings underscore the significance of the microbiota in *D. gallinae* and propose microbiota-targeted strategies for the management of mite populations on poultry farms [28].

### The general genetics of *D. gallinae*

The genus *Dermanyssus* comprises 25 species of haematophagous mites, which are morphologically divided into two subgenera: the hirsutus-group and Microdermanyssus, and the gallinae-group (to which *D. gallinae* belongs) [19]. Species in the first subgenus show many consistent morphological characters and host specificity. In contrast, species in the second subgenus are challenging to distinguish morphologically, and furthermore, due to their capacity to parasitise diverse avian species [14,29]. Research has identified the gene fragment for cytochrome c oxidase subunit I (COI) or 16S rRNA as the most effective genetic marker for studying the relatedness of *D. gallinae* [16,30–32]. The gene has been observed to demonstrate variability between geographically distant populations of *D. gallinae*, including across different countries or within countries. Conversely, studies have indicated that the internal transcribed spacer (ITS) fragment is the least useful genetic marker for studying the relatedness of *D. gallinae* populations, due to its excessive variability within populations [16,30]. The genetic variability of *D. gallinae* may have ramifications for its plasticity with respect to its host, resistance to environmental conditions and selection factors [14]. The acquisition of resistance by the mite to long-term and over-applied chemicals may be a consequence of genetic variability [4,15,33].

## The molecular basis of *D. gallinae* resistance to chemical acaricides

*D. gallinae* resistance to chemical acaricides is a global problem [33]. One of the commonly used agents was  $\beta$ -cypermethrin. Research has demonstrated that *D. gallinae* developed resistance to this substance [34–38]. Transmembrane proteins involved in the detoxification of xenobiotics are crucial in the resistance of *D. gallinae* to the applied miticides. In arthropods, the detoxification enzymes carboxylesterases (CarEs) are responsible for the detoxification of this substance. Research suggests that the mechanism for this resistance is increased activity of the CarEs protein and increased expression levels of the *Deg-CarE* gene. Zhang et al. [39] demonstrated that cypermethrin-resistant strains exhibited significantly higher *Deg-CarE* gene expression compared to  $\alpha$ -naphthyl acetate ( $\alpha$ -NA) and  $\beta$ -naphthyl acetate ( $\beta$ -NA)-resistant strains. Furthermore, *Deg-CarE* expression levels were found to be considerably higher in adults than in other developmental stages. In addition,  $\beta$ -cypermethrin has been observed to induce *Deg-CarE* expression. Silencing *Deg-CarE* gene by RNA interference decreased the activity of the enzyme and increased susceptibility to  $\beta$ -cypermethrin, confirming that *Deg-CarE* is crucial for  $\beta$ -cypermethrin detoxification. However, it has been shown that r*Deg-CarE* does not directly metabolise  $\beta$ -cypermethrin [39].

The ABC (ATP-binding cassette) family of transporter proteins has been identified as a significant component in the detoxification of xenobiotics [40,41]. Forty ABC transporters have been identified in the transcriptome and genome of *D. gallinae*. The results of this study indicate that five types of ABC transporters, the *DgABCA5*, *DgABCB4*, *DgABCD3*, *DgABCE1* and *DgABCG5* genes, may be associated with  $\beta$ -cypermethrin resistance in *D. gallinae* [42]. The transcription factor Cap 'n' Collar isoform-C (CncC) and its partner, fibromatosis of small muscle striatum (Maf), have been identified as key players in the mechanism of cypermethrin resistance [40]. The CncC function has been demonstrated to be implicated in the alleviation of oxidative stress [40]. The study demonstrated that these genes exhibited significantly higher expression levels in resistant strains compared to sensitive strains when exposed to beta-cypermethrin. Furthermore, the study revealed that the suppression of CncC/Maf pathway

genes through RNA interference (RNAi) resulted in the repression of ABC transporter genes, consequently enhancing the sensitivity of beta-cypermethrin-resistant strains [40]. This finding suggests that CncC/Maf plays a pivotal role in mediating *D. gallinae* resistance to beta-cypermethrin by regulating ABC transporters. Furthermore, it was observed that H<sub>2</sub>O<sub>2</sub> content and peroxidase (POD) and catalase (CAT) enzyme activities were significantly higher in resistant strains after beta-cypermethrin stress, indicating that beta-cypermethrin activates reactive oxygen species (ROS) [40]. In ROS scavenger assays, CncC/Maf expression was found to decrease significantly, along with a decrease in ABC transporter genes. Consequently, it can be concluded that the application of beta-cypermethrin instigates an outbreak of ROS, which subsequently activates the CncC/Maf pathway, resulting in the induction of ABC transporter-mediated drug resistance [40].

One strategy employed in the evaluation of control efficacy involves the identification of *VGSC* gene mutations as a means of addressing resistance. The presence of mite resistance to selected chemicals can be determined through the assessment of genetic markers. Associated with pyrethroid resistance, point mutations in the voltage-gated sodium channel gene (*VGSC*), particularly in the IIS4-IIS5 and IIIS6 fragments, have been observed. Four mutations within this gene have been demonstrated: I917V, M918T/L, A924G and L925V [43].

## Vaccines

The first protein to be selected and tested in a vaccine against *D. gallinae* was the somatic antigen of *D. gallinae* and other arthropods – a recombinant protein called subolesin (Subolesin SUB) and the Bm86 protein [44]. It was hypothesised that these would cause abnormalities in the parasite's development. The mortality rate of *D. gallinae* was 23% after Bm86 immunisation and 35% after subolesin immunisation [44]. Another trial looked at the cathepsin D-1 (Dg-CatD-1) protein [45]. The immunisation of hens with rDg-CatD-1 with Montanide™ ISA 71 VG was demonstrated to curtail the number of eggs laid by *D. gallinae* following a solitary blood draw [45]. The IgY class antibodies (anti-rDg-CatD-1) produced were found to be enduring in serum. Another protein, calumenin



(Deg-CALU), was also examined, and it was found to reduce the number of eggs laid by the mites by 35% [46]. A similar efficacy was demonstrated by Deg-AKR akirin, which resulted in a 42% reduction in the number of eggs laid after the mites ingested the blood of an immunised hen [47]. To date, no commercial vaccine against *D. gallinae* has been marketed.

### Integrated pest management (IPM)

IPM programmes have been shown to have a measurable effect on the control of mite populations [48]. IPM is a strategy that generally consists of an integrated approach to preventing and/or controlling organisms that are harmful to plants or animals by using all available information, tools and methods. It assumes the primacy of sustainable biological, physical and other non-chemical methods over chemical methods. The overarching objective of IPM is to effectively eradicate pests while minimising the reliance on chemical interventions and selecting methods that reduce risks to human health, beneficial and non-target organisms, and the environment, considering economic considerations. IPM emphasises the long-term prevention of pests or their damage through a combination of diverse methods and measures [48]. The utilisation of pesticides is contingent upon the findings of monitoring exercises, which are used to ascertain the necessity of such interventions in accordance with established guidelines. Treatments are executed with the express purpose of eradicating the target organism. The efficacy of specific measures can be ascertained through the screening for mutations of the VGSC gene, as previously described.

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