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Genetic Studies on Tick Ecdysteroid Regulation for Development of Sustainable Tick Control Strategies

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Ticks are exoparasites of mammals and important pests of livestock. Presently, tick control depends on use of acaricides and pesticides commonly used in integrated pest management. Despite a low efficacy for the control of ticks, these chemicals provide great advantages to livestock production. However, overuse results in the development of ticks resistant to these pest control agents. Therefore, development of alternative ways for sustainable livestock production is required. A knowledge of tick ecology and physiology is important for the development of strategies for integrated tick management. Tick molting and reproduction require blood feeding and are under endocrinological regulation. Ecdysteroids regulate tick molting and reproduction by combining with an ecdysteroid receptor (EcR). Ecdysteroid agonists are used in control of insects but these agonists are not effective against ticks. Studies on the regulation of molting and reproduction in ticks may provide effective tick control strategies leading to development of sustainable livestock production.

Key words: tick, ecdysteroid, arthropod growth regulator, ecdysteroid receptor, IPM

Introduction

Blood feeding arthropods directly cause great damage to livestock as well as transmit various diseases. Mosquitoes, flies and ticks are the major exoparasites of numerous host animals including livestock and humans (Peter *et al.,* 2005). Ticks transmit diseases such as tick theileriosis, anaplasmosis, babesiosis and tick fevers during blood feeding, these diseases greatly affect livestock production. Farmers and livestock industries mainly use acaricides to control ticks (George *et al.,* 2004). However, tick control with acaricides induces resistance in the pests (Bianchi *et al.,* 2003) so more effective and sustainable pest management techniques are required. To develop effective management, better understanding of the physiological processes that regulate reproduction in ticks is needed. In this paper, we introduce the characteristics of ticks and their importance as vectors of disease. Subsequently, we briefly review the present tick control techniques and then discuss endocrinological regulation of molting and reproduction induced by blood feeding as a possible strategy for tick control.

Tick Life Strategies

Ticks are blood feeding acari belonging to the class Arachnida and are classified into three families: Ixodidae, Argasidae and Nuttellidae. The most abundant species are hard ticks in the family Ixodidae, second are soft ticks in the family Argasidae and there is only one species of Nuttellidae. Hard and soft ticks occupy different habitats, and have different life cycles, feeding processes and reproductive strategies. Hard ticks live in grassy fields and go through one larval, one nymphal and an adult stage (Fig. 1). Hard ticks must obtain a blood meal three times during their life cycle, requiring almost 1 week for engorgement in females.

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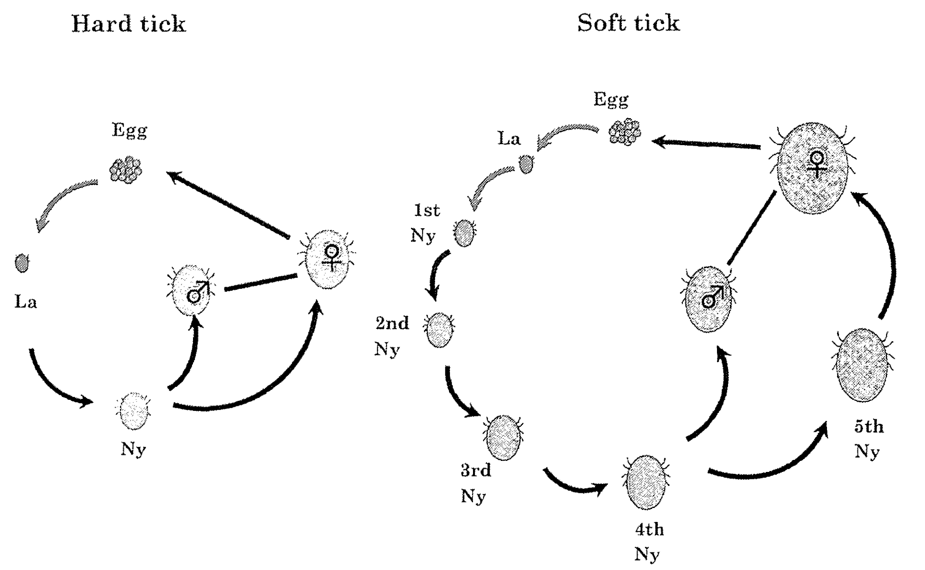

Fig. 1. Summary of hard and soft tick life cycles. La: larva, Ny: nymph. Gray arrows indicate molting without blood feeding from egg to larva. Black arrows indicate blood feeding is necessary before molting can occur.

Females of hard ticks mate on the host during blood feeding and die after egg laying. On the other hand, soft ticks live in the nests of small animals and have a more complex life cycle. Soft ticks go through one larval, several nymphal and an adult stage, and require a blood meal during all stages except for the larval stage in some species. Complete engorgement is achieved within one to two hours (Fig. 1). Argasid females can mate before or after blood feeding and can go through several reproductive cycles. The one species in the family Nutellidae shows characteristics similar both the ixodid and argasid ticks so it is placed in a separate family. Although all tick species depend on blood feeding for their development and reproduction, the ixodid and argasid ticks exhibit divergent life strategies.

Tick Damage and Control

Blood feeding causes severe damage to livestock by the induction of anemia and appetite loss that weakens livestock and reduces yield (reviewed by Jonsson, 2006). In addition, bite marks of ticks greatly decrease the value of the hide. Ticks secrete substances from their salivary glands into the host animals to expedite blood feeding. Sometimes this saliva contains toxins or pathogens that cause tick paralysis resulting in extreme fevers and death of the host. Ticks can transmit various pathogens including bacteria, viruses, protozoans and nematodes. Tropical theileria is an important disease of livestock production in Asia caused by babesia a protozoan transmitted by ticks (Ahmed *et al.*, 2008). Other tick borne diseases (TBDs) also cause severe damage to livestock production. Anaplasmosis, which sometimes occurs as a mixed infection with other TBDs, is the most severe disease in South Africa with more than 99% of cattle at risk of infection (de Waal, 2000; Mayo and Masika, 2008). Costs for control of TBD and economic losses in global cattle production were estimated to be 13.9 and 18.9 billion per year (de Castro, 1997). The losses in livestock production caused by ticks are indeed economically significant on a worldwide basis. In addition, the distribution of ticks is expected to expand as global warming continues (Ogeden *et al.*, 2008). Development of effective and sustainable strategies for tick control is becoming more and more important.

New alternatives are needed for sustainable tick control to prevent direct damage and disease transmission to livestock. Presently, acaricides are the major methods used for tick control (George *et al.*, 2004) but induce acaricide-resistance and may contaminate food and the environment (Taylor, 2001; Bianch *et al.*, 2003; Graf *et al.*, 2004). Several vaccines useful to prevent livestock from contracting particular diseases such as tick theileriosis have been developed. However, ticks transmit numerous pathogens so the development of better and unique vaccines is required (de la Fuente *et al.,* 2007). Arthropod growth regulators (AGR) that interrupt the usual development of arthropods and chitinase inhibitors that interrupt establishment of the arthropod cuticle are also somewhat effective for tick control. Analogues of arthropod hormones are used for management of insects, but these chemicals are not presently effective for control of ticks. To develop effective acaricides or vaccines, understanding tick physiological processes, particularly the hormonal regulation of molting and reproduction, is essential. Therefore, we focus our research on the hormonal regulation of reproduction in the soft tick *Ornithodoros moubata* in order to contribute to the development of novel strategies for tick control.

Endocrinology of Tick Molting and Reproduction

Insects, crustaceans and arachnids (spiders, ticks and scorpions) are likely sister groups and have similar endocrinological regulatory mechanisms for development and reproduction. Based on insect studies, two hormone groups the juvenoids and molting hormones appear to provide critical cues for development and reproduction. Juvenile hormone (JH) is a juvenoid that regulates insect growth and reproduction. JH modifies the functions of molting hormones and regulates larval-larval molting of immature stages and also egg production of most insects (Riddiford, 1996). Crustaceans utilize methyl farnesoate a precursor of JH for molting and reproduction (LeBlanc, 2007). Functions of JH and JH analogues in ticks have been studied by several researchers with conflicting results. Early studies on JH in ticks used exogenous JH and JH analogues and showed that treatment with JH and JH analogues induced egg production and oviposition in fed virgin females of *Ornithodoros moubata* and *Ornithodoros parkeri* (Connat *et al.,* 1983; Pound and Oliver, 1979). However, Chinzei *et al.* (1991) and Taylor *et al.* (1991) showed no stimulation of yolk protein synthesis in the same species. Neese *et al.* (2000) examined biosynthesis of JH in ticks and could not detect synthesize or activity of JH, JH precursors and farnesoates in the nymphal and adult stages of either hard or soft ticks. Therefore, recent studies indicate ticks do not have JH so juvenoids do not appear to function in the regulation of development and reproduction of ticks.

Molting hormones (ecdysteroids) are common in many arthropod species. Because arthropods can not biosynthesis the skeletal structure of steroids, ecdysteroids are derived from the food of arthropods (Chapman, 1998). Ecdysteroids provide critical cues for molting and metamorphosis. Increases in ecdysteroid titers appear in arthropod hemolymph before ecdysis. Both hard and soft ticks also release ecdysteroids for molting (reviewed in Rees, 2004). Egg production of dipteran insects including flies and mosquitoes are regulated by ecdysteroids. Egg production is also regulated by ecdysteroids in ticks (reviewed in Taylor and Chinzei, 2002). This indicates ecdysteroids are essential hormones for the regulation of tick development and reproduction.

Molecular Regulation of Tick Molting and Reproduction

Although hard and soft ticks have different survival strategies, molting and reproduction in both ticks are regulated by similar endocrinological mechanisms. Ecdysteroids regulate molting and reproduction through gene transcription. Ecdysteroids function by binding to a functional heterodimer receptor formed from the ecdysteroid receptor (EcR) and retinoid X receptor (RXR). Ecdysteroid, EcR and RXR form a complex and bind to the promoter region of target genes to regulate gene expression (Yao *et al.,* 1992; Yao *et al.,* 1993; Thomas *et al.,* 1993). EcRs are members of the nuclear receptor family with a common molecular structure. EcR and RXR have been determined from the hard tick *Amblyomma americanum* (Guo *et al.,* 1997; Guo *et al.,* 1998) and the soft tick *Ornithodoros moubata* (Horigane *et al.,* 2007, 2008). Ecdysteroid titers increase in the last instar nymphs after blood feeding in both hard and soft ticks (Palmer *et al.,* 2002; Ogihara *et al.,* 2007). *O. moubata* EcR and RXR expression also increases immediately after blood feeding (Horigane *et al.,* 2007; Horigane *et al.,* 2008). EcR of *AamEcR*s is also expressed after blood feeding with high titers

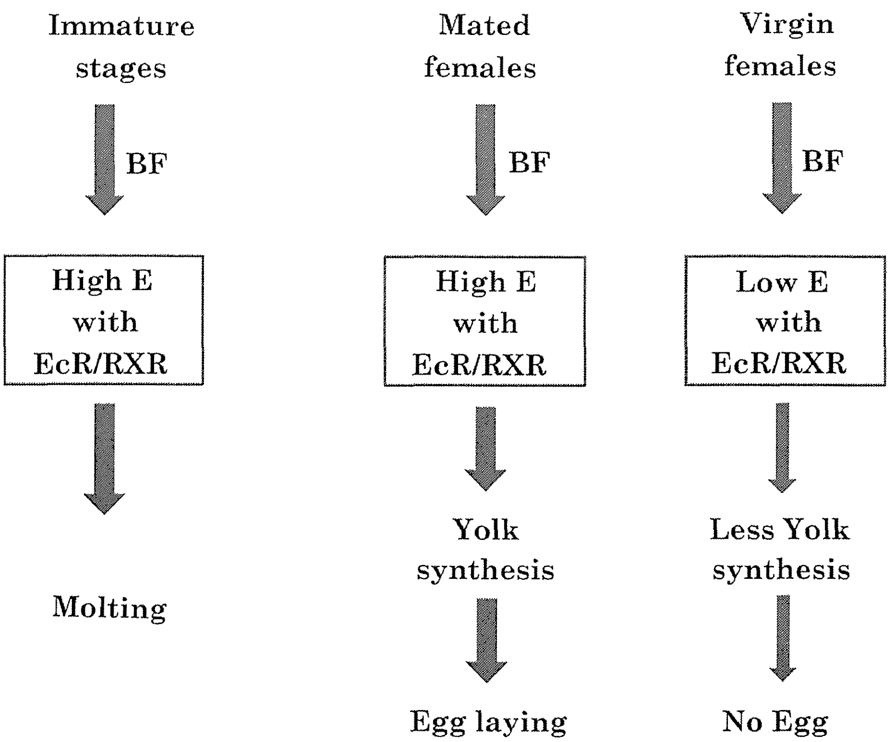

Fig. 2. Outline of molting and egg production of ticks after blood feeding. BF: blood feeding, E: ecdysteroids

of ecdysteroids during molting in larval and nymphal stages (Palmer *et al.*, 2002). Therefore, EcR and RXR expression in ticks coincides with high titers of ecdysteroids during molting and after blood feeding.

Ecdysteroid release is also observed in adult female ticks after blood feeding. Female hard ticks must mate on the host animal during blood feeding because mated females enter a rapid feeding phase during which they become fully engorged. Ecdysteroid titers in the hemolymph of *A. hebraeum* increase during and after blood feeding (Mao and Kaufman, 1998). Increases in EcR proteins of the hard tick *A. hebraeum* also occur after blood feeding (Mao and Kaufman, 1998). Soft ticks can mate before and after blood feeding. Females of *O. moubata* show increases of EcR and RXR expression after blood feeding in both mated and virgin females (Horigane *et al.*, 2007; Horigane *et al.*, 2008). On the other hand, ecdysteroid titers in the hemolymph increase only in mated females but not in virgin females (Ogihara *et al.*, 2007). Although both mated and virgin females feed similarly, only mated females can synthesize sufficient yolk protein concentrations to produce mature eggs (Ogihara *et al.*, 2007) because only mated females have high titers of ecdysteroids that coincide with EcR and RXR expression (Fig. 2). Therefore, ecdysteroids require EcR and RXR to function and are essential hormones for tick development and reproduction.

Conclusions and Recommendations for Integrated tick Management

Genetic analysis provides basic knowledge about the regulation of molting and reproduction in arthropods and may lead to the development of tools for tick control. Insect growth hormones such as JH and ecdysteroids are used as insect growth regulators (IGR) to effectively control some species but these regulators are not effective for tick control. The only hormones confirmed in ticks are ecdysteroids, so the target for development of a growth regulator for ticks is ecdysteroids. EcR has been identified from several insect species, ticks and crustaceans and shown to have conserved sequences and functions. However, the ligand binding domain (LBD), target site for ecdysteroid agonists or antagonists, shows a variety of differences among arthropod species (Bonneton *et al.,* 2003). EcR of the soft tick *O. moubata* (OmEcR) showed only 58% identities to moth EcRs (Horigane *et al.,* 2007). The differences in the EcR LBD structure are related to selectivity of ecdysteroid agonists in insects (Billas *et al.,* 2003; Carmicaher *et al.,* 2005). Diacylhydrozines (DAH) are non-steroidal ecdysteroid agonists used to control Lepidoptera but these agonists show low efficacy toward Hemiptera (Billas *et al.,* 2003). This different effect is likely due to the LBD structure of EcR. Tick EcRs may have different affinities for ecdysteroid analogues because the LBD of ticks differ from other arthropods. In addition, EcR of mites are expected to be similar to ticks. Therefore, understanding the structure of this receptor can lead to the development of specific ecdysteroid agonists and antagonists for tick and mite control. The control of development and reproduction in ticks and mites by ecdysteroid analogues may decrease acari borne diseases and damage leading to the development of more sustainable livestock and crop production.

References

Ahmed, J.S., Glass, E.J., Salih, D.A. and Seitzer, U., 2008. Innate immunity to tropical theileriosis. Innate Immun. 14, 5-12.

Bonneton, F., Zelus, D., Iwema, T., Robinson-Rechavi, M. and Laudet, V., 2003. Rapid divergence of the ecdysone receptor in Diptera and Lepidoptera suggests coevolution between ECR and USP-RXR. Mol. Biol. Evol. 20, 541-553.

Billas, I.M., Iwema, T., Garnier, J.M., Mitschler, A., Rochel, N. and Moras, D., 2003. Structural adaptability in the ligand-binding pocket of the ecdysone hormone receptor. Nature 426, 91-96.

Carmichael, J.A., Lawrence, M.C., Graham, L.D., Pilling, P.A., Epa, V.C., Noyce, L., Lovrecz, G., Winkler, D. A., Pawlak Skrzecz, A., Eaton, R.E., Hannan, G.N. and Hill, R.J., 2005. The X-ray structure of a hemipteran ecdysone receptor ligand-binding domain: comparison with a lepidopteran ecdysone receptor ligand-binding domain and implications for insecticide design. J. Biol. Chem. 280, 22258-22269.

Chapman, R.F., 1998. Endocrine system, Chapter 21. In: The Insects. Cambridge University Press., New York (NY), pp. 570-584.

Chinzei, Y., Taylor, D. and Ando, K., 1991. Effects of juvenile hormone and its analogs on vitellogenin synthesis and ovarian development in the soft tick, *Ornithodoros moubata* (Acari: Argasidae). J. Med. Entomol. 28, 506-513.

de Castro, J.J., 1997. Sustainable tick and tickborne disease control in livestock improvement in developing countries. Vet. Parasitol. 71, 77-97.

de la Fuente, J., Kocan, K.M. and Blouin, E.F., 2007. Tick vaccines and the transmission of tick-borne pathogens. Vet. Res. Commun. 31 Suppl 1, 85-90.

de Waal, D.T., 2000. Anaplasmosis control and diagnosis in South Africa. Ann. NY Acad. Sci. 916, 474-483.

George, J.E., Pound, J.M. and Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129 Suppl, S353-366.

Graf, J.F., Gogolewski, R., Leach Bing, N., Sabatini, G.A., Molento, M.B., Bordin, E.L. and Arantes, G.J., 2004. Tick control: an industry point of view. Parasitology 129 Suppl: S427-442.

Guo, X., Harmon, M.A., Laudet, V., Mangelsdorf, D.J. and Palmer, M.J., 1997. Isolation of a functional ecdysteroid receptor homologue from the ixodid tick *Amblyomma americanum* (L.). Insect Biochem. Mol. Biol. 27, 945-962.

Guo, X., Xu, Q., Harmon, M.A., Jin, X., Laudet, V., Mangelsdorf, D.J. and Palmer, M.J., 1998. Isolation of two functional retinoid X receptor subtypes from the Ixodid tick, *Amblyomma americanum* (L.). Mol. Cell. Endocrinol. 139, 45-60.

Horigane, M., Ogihara, K., Nakajima, Y., Shinoda, T. and Taylor, D., 2007. Cloning and expression of the ecdysteroid receptor during ecdysis and reproduction in females of the soft tick, *Ornithodoros moubata* (Acari: Argasidae). Insect Mol. Biol. 16, 601-612.

Horigane, M., Ogihara, K., Nakajima, Y. and Taylor, D., 2008. Isolation and expression of the retinoid X receptor from last instar nymphs and adult females of the soft tick *Ornithodoros moubata* (Acari: Argasidae). Gen. Comp. Endocrinol. 156, 298-311.

Jonsson, N.N., Bock, R.E. and Jorgensen, W.K., 2008. Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses, and the effects of differing intensity of tick control in Australia. Vet. Parasitol. 155, 1-9.

LeBlanc, G.A., 2007. Crustacean endocrine toxicology: a review. Ecotoxicology 16, 61-81.

Mao, H. and Kaufman, W.R., 1999. Profile of the ecdysteroid hormone and its receptor in the salivary gland of the adult female tick, *Amblyomma hebraeum*. Insect Biochem. Mol. Biol. 29, 33-42.

Moyo, B. and Masika, P.J., 2008. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Trop. Anim. Health Prod. (Online 2008 Aug 14).

Neese, P.A., Sonenshine D.E., Kallapur, V.L., Apperson, C. S. and Roe, R.M., 2000. Absence of insect juvenile hormones in the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae), and in *Ornithodoros parkeri* Cooley (Acari: Argasidae). J. Insect Physiol. 46, 477-490.

Ogden, N.H., St Onge, L., Barker, I.K., Brazeau, S., Bigras Poulin, M., Charron, D.F., Francis, C.M., Heagy, A., Lindsay, L.R., Maarouf, A., Michel, P., Milord, F., O'Callaghan, C.J., Trudel, L. and Thompson, R.A., 2008. Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis,* in Canada now and with climate change. Int. J. Health Geogr. 7, 24.

Ogihara, K., Horigane, M., Nakajima, Y., Moribayashi, A. and Taylor, D., 2007. Ecdysteroid hormone titer and its relationship to vitellogenesis in the soft tick, *Ornithodoros moubata* (Acari: Argasidae). Gen. Comp. Endocrinol. 150, 371-380.

Palmer, M.J., Warren, J.T., Jin, X., Guo, X. and Gilbert, L. I., 2002. Developmental profiles of ecdysteroids, ecdysteroid receptor mRNAs and DNA binding properties of ecdysteroid receptors in the Ixodid tick *Amblyomma americanum* (L.). Insect Biochem. Mol. Biol. 32, 465-476.

Peter, R.J., Van den Bossche, P., Penzhorn, B.L. and Sharp, B., 2005. Tick, fly, and mosquito control-lessons from the past, solutions for the future. Vet. Parasitol. 132, 205-215.

Pound, J.M. and Oliver, J.H., Jr., 1979. Juvenile hormone: Evidence of its role in the reproduction of ticks. Science 206, 355-357.

Rees, H.H., 2004. Hormonal control of tick development and reproduction. Parasitology 129 Suppl, S127-143.

Riddiford, L.M., 1996. Juvenile hormone: the status of its "status quo" action. Arch. Insect Biochem. Physiol. 32, 271-286.

Taylor, D., Chinzei, Y. and Ando, K., 1991. Vitellogenin synthesis, processing and hormonal regulation in the tick, *Ornithodoros parkeri* (Acari: Argasidae). Insect Biochem. 21, 723-733.

Taylor, M.A., 2001. Recent developments in ectoparasiticides. Vet. J. 161, 253-268.

Taylor, D. and Chinzei, Y., 2002. Vitellogenesis in Ticks, Chapter 6. In: Raikhel, A.S., Sappington, T.W. (Eds.), Reproductive Biology of Invertebrates Volume XII — Recent Progress in Vitellogenesis. Science Publishers Inc., Enfield (NH), pp. 175-199.

Thomas, H.E., Stunnenberg, H.G. and Stewart, A.F., 1993. Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and ultraspiracle. Nature 362, 471-475.

Tobe, S.S. and Bendena, W.G., 1999. The regulation of juvenile hormone production in arthropods. Functional and evolutionary perspectives. Ann. NY Acad. Sci. 897, 300-310.

Yao, T.P., Segraves, W.A., Oro, A.E., McKeown, M. and Evans, R.M., 1992. *Drosophila* ultraspiracle modulates ecdysone receptor function via heterodimer formation. Cell 71, 63-72.

Yao, T.P., Forman, B.M., Jiang, Z., Cherbas, L., Chen, J. D., McKeown, M., Cherbas, P. and Evans, R.M., 1993. Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. Nature 366, 476-479.