Isolation of the Ecdysteroid Receptor and Retinoid X Receptor from the Spider *Agelena silvatica*

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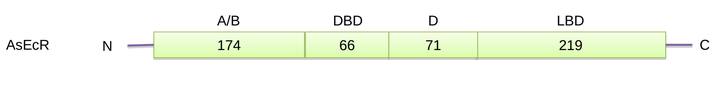
Introduction

For growth and development, arthropods must repeat several molts that involve complicated process such as new cuticle synthesis, old cuticle resolution and apolysis. These processes are mainly regulated by ecdysteroids and juvenile hormone. A model for the regulation of gene transcription by ecdysteroids and the ecdysone receptor (EcR) has been developed in insects (Fig. 3). EcR becomes functional for ligand binding and DNA binding by forming a heterodimer with ultraspiracle (USP). Some primitive insects and other arthropods use RXR instead of USP. EcR and USP or RXR have been identified in insects, crustaceans, ticks and scorpions. These reports indicate the basic mechanisms involving ecdysteroids and their transcriptional factors are common among arthropods. However, some differences occur in the receptors for each arthropod group. Spiders are well known, but there are only a few reports on the endocrinology of spiders, and EcR and RXR have not been identified from spiders despite identification in closely related orders such as the Acari and Scorpionida. Therefore, we isolated AsEcR and AsRXR from the spider *Agelena silvatica* and compared these receptors with EcRs and USPs or RXRs from other animals to determine the role of ecdysteroids in the regulation of molting of spiders and to better understand the evolution and functions of EcR and RXR in arthropods. As far as we know, this is the first report of hormone receptors from spiders.

EcR

A



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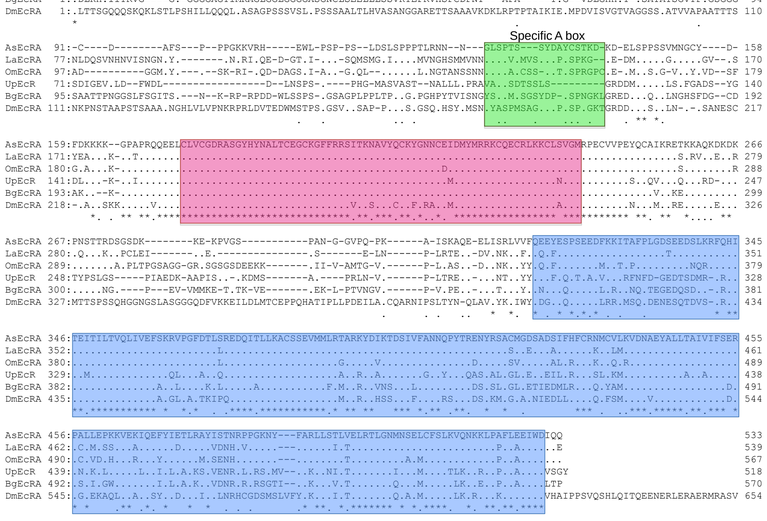


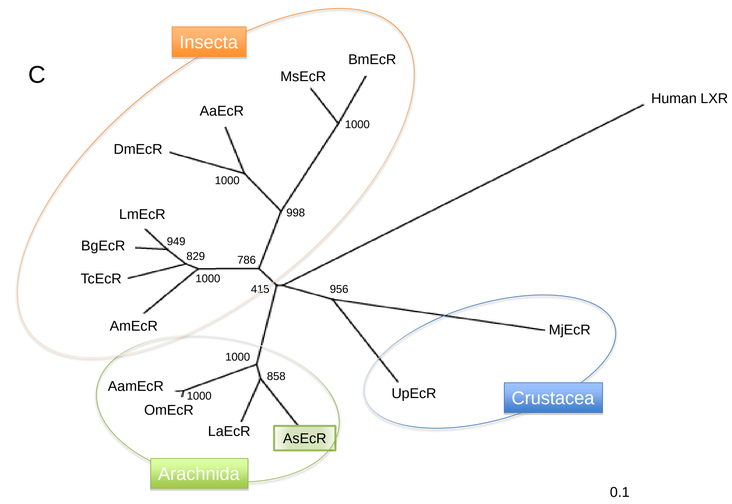
Fig. 1. (A) Schematic of isolated AsEcR. AsEcR has the conserved nuclear receptor structure. Numbers show the amino acids for each domain. (B) Amino acid alignment of AsEcRA with other arthropod EcRs. EcR genes for alignment include: LaEcR from *Liocheles australasiae* (AB297929), OmEcRA from *Ornithodoros moubata* (AB191193), UpEcR from *Uca pugilator* (AF034086), BgEcRA from *Blattella germanica* (AM039690) and DmEcRA from *Drosophila melanogaster* (S63761). The green box indicates specific A box nucleotide sequence characteristic of the EcRA isoform. The pink box indicates the DNA binding domain and the blue box the ligand binding domain. Dots indicate the same amino acid as the sequence above. (C) Phylogenetic tree of the Ligand Binding Domain for EcRs. AsEcR is included in the Arachnida group.

Table 1. Homology of AsEcRA compaired with other EcRs

Amino acids		% Identity (similarity) of each domain A/B	C	D	E
LaEcRA	Scorpion	40 (50)	100 (100)	55 (69)	89 (94)
OmEcRA	Tick	42 (53)	99 (100)	56 (69)	84 (91)
UpEcR	Crab	36 (48)	97 (100)	43 (61)	66 (80)
MjEcRA	Prawn	22 (30)	96 (100)	32 (37)	57 (73)
BgEcRA	Orthoptera	26 (31)	100 (100)	56 (66)	66 (80)
TcEcRA	Coleoptera	19 (31)	100 (100)	55 (61)	69 (80)
AmEcRA	Hymenoptera	30 (38)	99 (100)	43 (51)	70 (80)
MsEcRA	Lepidoptera	35 (44)	89 (97)	33 (44)	58 (71)
MsEcRB1		27 (38)			
DmEcRA	Diptera	30 (38)	88 (96)	38 (50)	61 (77)
DmEcRB1		36 (47)			

The homologies of each domain were determined by EMBOSS needle program. Some EcRs used are explained in Fig.1B and the additional EcRs used for homology comparison include: MjEcRA from *Marsupenaeus japonicus* (AB295492), TcEcRA from *Tribolium castaneum* (AM295015), AmEcRA from *Apis mellifera* (NM_001098215), MsEcRA from *Manduca sexta* (U49246), MsEcRB1 (U19812), DmEcRB1 from *Drosophila melanogaster* (M74078).The C domain (DBD) and E domain (LBD) showed high identities with other EcRs. In the DBD, scorpion, cockroach and flour beetle showed 100% identities with AsEcRA. The LBD showed higher identities with other arachnida, scorpion and soft tick.

C



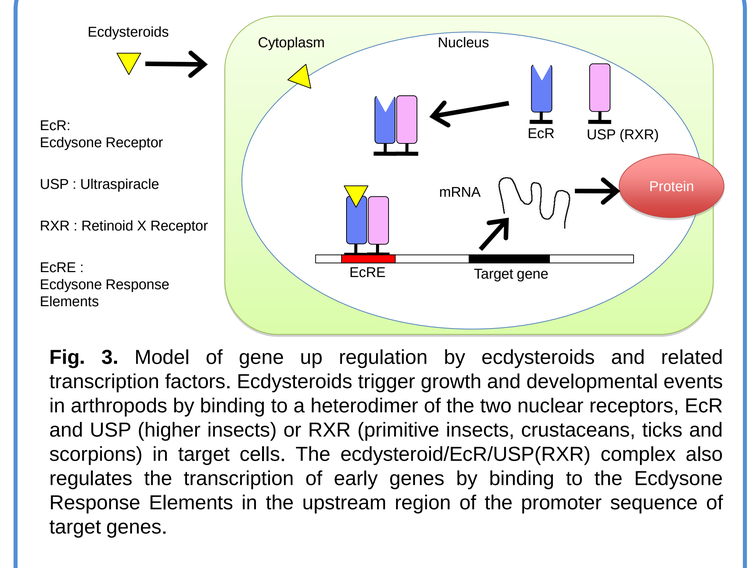


Fig. 3. Model of gene up regulation by ecdysteroids and related transcription factors. Ecdysteroids trigger growth and developmental events in arthropods by binding to a heterodimer of the two nuclear receptors, EcR and USP (higher insects) or RXR (primitive insects, crustaceans, ticks and scorpions) in target cells. The ecdysteroid/EcR/USP(RXR) complex also regulates the transcription of early genes by binding to the Ecdysone Response Elements in the upstream region of the promoter sequence of target genes.

Fig. 4. Model of nuclear receptor sequence domains. Nuclear receptors are divided to five domains based on functions. The N terminal A/B domain functions in transcriptional activation and EcR isoforms possess different A/B domains. The central C domain is the DNA binding domain (DBD) and includes two zinc fingers to recognize specific sequences in the upstream region of target genes. The D domain functions as a hinge for the C and E/F domains. The E domain is the ligand binding domain (LBD) constructed from 10-12 α-helices that forms a hydrophobic ligand-binding pocket. The DBD and LBD are highly conserved in all nuclear receptors.

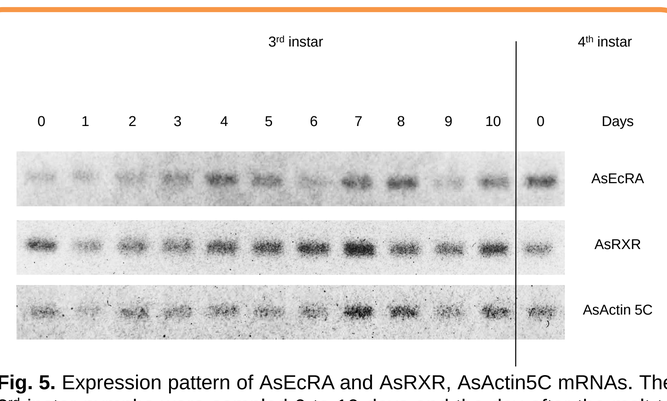
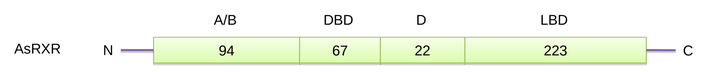


Fig. 5. Expression pattern of AsEcRA and AsRXR, AsActin5C mRNAs. The 3rd instar nymphs were sampled 0 to 10 days and the day after the molt to the 4th instar. The experiment was repeated three times using different individuals. AsActin 5C used as a control was also determined in this study (data not shown). AsEcRA and AsRXR were constantly expressed every day before molting and after molting.

RXR

A



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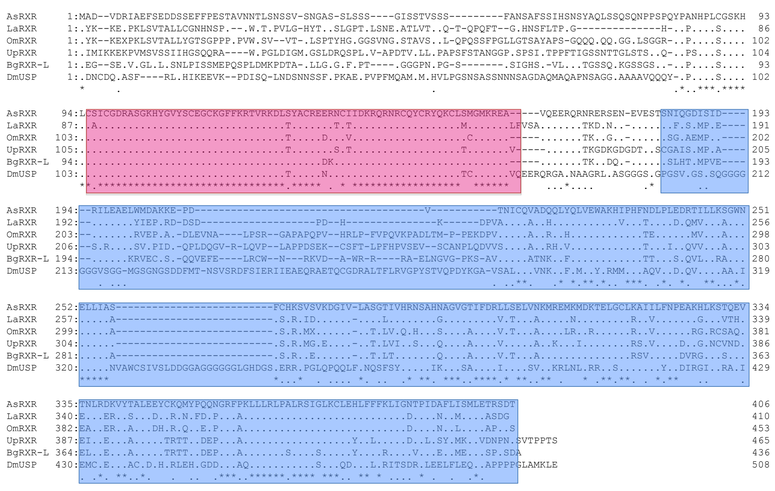


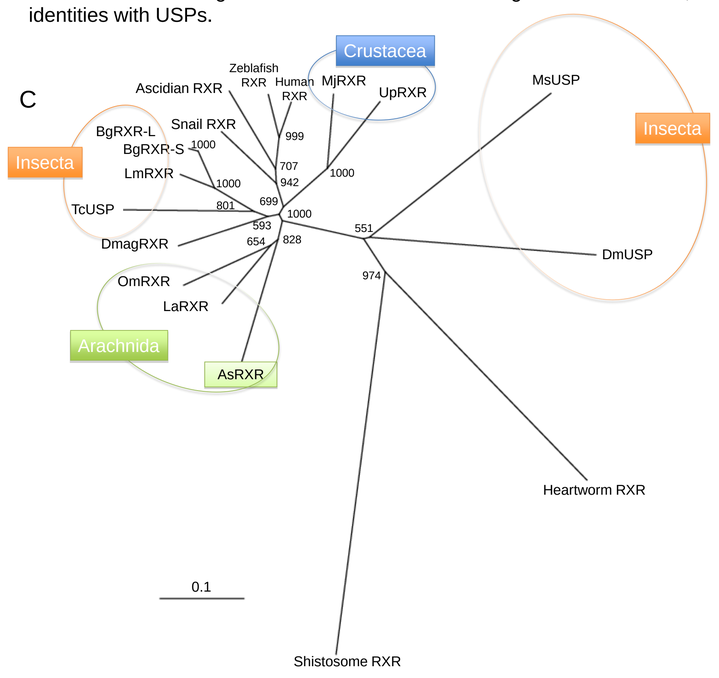
Fig. 2. (A) Schematic of isolated AsRXR. AsRXR has the conserved structure of nuclear receptors. Numbers show the amino acids for each domain. (B) Amino acid alignment of AsRXR with other arthropods RXRs and USPs. Other RXR and USP genes include: LaEcR from *Liocheles australasiae* (AB297930), OmRXR from *Ornithodoros moubata* (AB353290), UpRXR from *Uca pugilator* (AF032983), BgRXR-L from *Blattella germanica* (AJ854490) and DmUSP from *Drosophila melanogaster* (NM_057433). The pink box indicates the DNA binding domain and the blue box the ligand binding domain. Dots indicate the same amino acid as the sequence above. (C) Phylogenetic tree of the Ligand Binding Domain of RXRs and USPs. AsRXR is included in the Arachnida group and widely separated from USPs.

Table 2. Homology of AsRXR compaired with other RXRs and USPs

Amino acids		% Identity (similarity) of each domain A/B	C	D	E
LaRXR	Scorpion	37 (53)	93 (96)	80 (95)	74 (85)
OmRXR	Tick	38 (51)	94 (99)	86 (91)	61 (75)
UpRXR	Crab	31 (47)	94 (99)	55 (77)	57 (69)
MjRXR	Prawn	43 (55)	96 (97)	42 (63)	66 (80)
BgRXR-S	Orthoptera	33 (42)	97 (100)	82 (91)	66 (80)
TcUSP	Coleoptera	37 (45)	97 (100)	73 (86)	58 (76)
AmUSP	Hymenoptera	35 (43)	96 (100)	77 (91)	66 (81)
MsUSP	Lepidoptera	58 (62)	94 (99)	65 (77)	41 (60)
DmUSP	Diptera	33 (48)	94 (97)	46 (64)	40 (58)
HsRXR alfa	Human	27 (36)	85 (97)	82 (96)	66 (81)

The homologies of each domain were determined by EMBOSS needle program. The RXRs and USPs used are explained in Fig. 2B and also include: MjRXR from *Marsupenaeus japonicus* (AB295493), TcUSP from *Tribolium castaneum* (NM_001114294), AmUSP from *Apis mellifera* (NM_001011634), MsUSP from *Manduca sexta* (MSU44837), DmUSP from *Drosophila melanogaster* (NM_057433) .The C domain (DBD) and E domain (LBD) showed high identities with all other sequences. The DBD showed high identities with other arthropods. The LBD showed high identities with RXRs including the Human RXR, but low identities with USPs.

C



Conclusion

① AsEcR and AsRXR sequences were identified and shown to have the characteristics conserved in nuclear receptors.
② The DBD and LBD of both AsEcR and AsRXR showed high identities with ticks and scorpions.

These results suggest that AsEcR and AsRXR function as nuclear receptors for the regulation of molting in spiders as seen in other arthropods.

The phylogenetic trees and identities of the LBD indicate the ligand response of arachnid EcR and RXR may differ from insects.

Materials and Methods

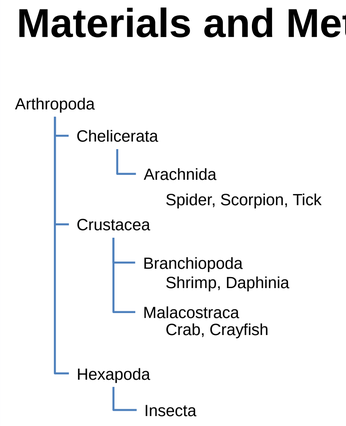


Fig. 6. Phylogeny of arthropods. Spider belong to Chelicerata different to crustaceans or insects.

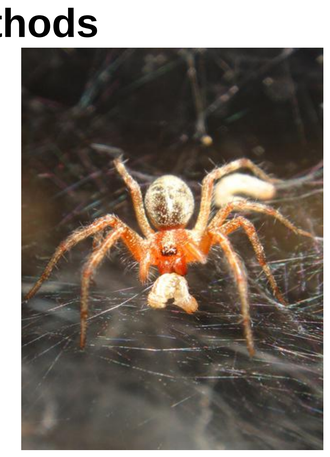


Fig. 7. A nymph of *Agelena silvatica*. All specimens used in this study were collected from the University of Tsukuba campus, Tsukuba, Japan.

Materials

Spiders are classified as Arachnida, which also includes ticks and scorpions. Spiders make webs from spider silk to capture terrestrial arthopods for food. Spiders are gaining attention as natural enemies for environmentally friendly biocontrol agents in agriculture. *Agelena silvatica* was selected for this study because ecdysteroid titers were previously determined from *Coelotes terrestris* and *Tegenaria atrica* species in the same family (Trabalon et al., 1992), and *A. silvatica* is very common in Japan so could be easily colleted on the University of Tsukuba Campus. *A. silvatica* constructs a large random web sheet on camellia and other plants. The adult female reaches about 17 mm in length and the male about 14 mm. We collected egg sacs of this species and used the eggs or nymphs that emerged from the egg sacs.

Methods

RNA was extracted from eggs of *A. silvatica,* degenerate primers were designed based on scorpion EcRs and RXRs. RACE was used to determine the full length of cDNA. Expression analysis was carried out by RT-PCR on 3rd instar nymphs.

Reference

Trabalon, M., et al., 1992. *Gen. Comp. Endocrinol*. 88, 128-136.