

RNAi-mediated knockdown of transcription factor E93 in nymphs of the desert locust (*Schistocerca gregaria*) inhibits adult morphogenesis and results in supernumerary juvenile stages

Marijke Gijbels, Elisabeth Marchal[§], Thomas Wolf Verdonckt, Evert Bruyninckx, Jozef Vanden Broeck*

Research group of Molecular Developmental Physiology and Signal Transduction, KU Leuven, Zoological Institute, Naamsestraat 59 box 2465, 3000 Leuven, Belgium

[§]Current address: Life Science Technologies, Imec, Kapeldreef 75, B- 3001 Leuven, Belgium

*Correspondence:

Jozef Vanden Broeck: jozef.vandenbroeck@kuleuven.be

Marijke.Gijbels@kuleuven.be

Elisabeth.Marchal@imec.be

ThomasWolf.Verdonckt@kuleuven.be

Evert.Bruyninckx@kuleuven.be

Jozef.VandenBroeck@kuleuven.be

ATGGCGGAGTGCTCCTATGCTCGCTGTGTTTCAGGAGCGCCGAGCAATCAAAAAAGAGCTGCAAAGATGGACG
AAGAATATGGTTTTCGTAGTAGTTTGAACGGGTAGCGGAGGAGTTAATGGGCAGAAGGAAATGGAAACA
TTATCAGGAGTCAGTTTTGAGAAGTAGACCGCAAGCAGACATAGAACCCAGCAGCAGTGATTGGCAAGTTGA
AGACAAATGCTGCTTTTGCACGCGTGGGGATGTTCTCTGAGACCAAGCAACAAGTCCACAGTCAGACAGCTGCAGT
AGTTCCAACAGCCACAGCAGTTTTACGAACTCAGTCGACAGTAGCAGCAGACAGTTCCTCCAGGCTGG
CGATGACAACACTCGAACCGGTCACGTCGCTGGCCGCGTCACTGGCAGCTGTCCCGACCTACAGCCCGGACC
CGCACAGGGGCACTCCGCAATGGCAACCAACCCCTCCAGCAGGCCTCTCTCTACCCACAGTAGCACGCCATC
GGACACCTGCGGGACTCTTCCCTCCGTGGTACCTGTCACCGACGGCACCTGCTCCGAATGTTTCGATCGGAAGG
AAAGACTGAACCGGAACTCCACCCGTCGTCGTATCCCTCCCTGCATCTACACCGCTGTCGGTGCCGTCAGCTG
CGGCGAGTGAGCAGCCTTTGGACTTGAGTGCCAAAGCCAAGGAAGAAGCATCTGCACCCAGCACCTCACCCGT
GCCTCAGCCTTTGGGATTGGCACTTTGAGTTTGGACAGCAAGCAGATATTCAAGGCGAAACCAAGAATGAGT
GCAGTGGCAGGTGCAAGGACATACACAGAAGAAGAGCTTCAAGCTGCTCTGAGAGATATCCAGAGTGGAAAA
TTAGGAACCTCGGCGTGACGCTGTTATTTATGGTATACCACGCTCCACACTTCGGAACAAAGTGTACAAGTTGGC
TATGGAGCGTGAACGTGACGCACACTTAGTGTTCTACAGTAGAGGAAAGAGACCTGTCTGGCGCTGAGGA
TGAGAAGGAAGTTGAACGTGCTTTAAGTCGTCTCTCTTCTGTTGACGATCTTCTGCGATTATCTGTGCTAGA
TGGGGATGCTCTTAGAGTTCTTCTGGAGGAAGTTTCTGCAGGACACAATCGTGAAGCAAGTGAGTCAGCATCT
TCCCCAGTGTTCACAATCTAGTGAACCTTTGGCATGGTCTAGAACAGAGTGCATTGGGACCTTACATCTCCCA
GCTTCTGATAGCTGGAAGTGCTCAAGATCCTTGCCATGCTGAAAGCGGAGGTACACTCCCAAAATTTACTTCCC
CTCTTCTCCCCGAACCTGTCCGTCGCATGATGGCAGAGGAGCAGCAACAGCAGATAAAGAAGCTAGGTAGTGC
CAATAATTTGGAAGCAAATACAAATATAACATTGAATGGTGCTTCAGGTGATGAGATACAGAAGTTAGGTGAT
AGCGACAACAGTGCTGGGCCACCGAGAGTTCCATCTGCATCATCTCAAACAGACCCTGTGAAAGCTACAACAA
AAGTTGGGGAAGCAGATGAATCATCTGACACAGATGCAACAGCTTCACTTCTAGAGTTGCCACACCACCAAA
TGTGATACTGAGAATTCCGTCCTTTAAACCAACTACGAAAAATGGTGTGGCTACTCCAGGAAGTGGAAGTTTG
CCTTCTAGTGAAATTCATTCCAGACTGTTGGAGTTCCTGCATTTCTGGCCAGGAATCTAGTCATGTATTGAAC
AGCAGCAGTAACAATAGTAATGAGTCTTGTTACCACCAGCATCTAACCTTGAGGAAAAGGGATTGGGGTCA
GTTTACGTGATGTTATAGCCAAAAGCATTAGTCAGAAGTTTCAACCAACTGATCTTCAATGTCACAGAAGCTC

TTATTATCAGCAGATGAACAACCCCATTTAAGAGAGGTCGTTTTACTCCTCCATTAAATACAAATATCACATCG
TGCATAATAAACACAATAACAATAACAACACTAATGTTGATGATAAAAAACAAGACATCCTCTGCAGGTGGAA
AACTTCATCAAATTCTACTGGCAAAGGAACCCGGCCAAAACGTGGAAAATACCGTAATTATGATCGTGATAG
TTTGGTAGAAGCTGTTAGAGCAGTGCAGAGGGGTGAAATGAGTGTCCATCGGGCAGGATCATATTATGGTGT
TCCACATTCAACATTAGAATACAAGGTGAAAGAGAGACATTTGATGAGACCTCGGAAGAGGGA**GCCCAAGCA**
ACAACAGTCTGATGAAGTTAAAAGGAAAGATGAATCTGGGACTTCAATTCCAAGGAGTACAGCAACAACCTCA
TCAGCACTGGCAAATGATAAACTTAAACTTTGCCTAAACCTCCCAAGACTGCCTTTAATTCACCTGCTCCATTA
CCTGGTGCACCAAATGGTCTGAAAATACCACCCTTGTGGTATCCCAGTTTATCTCTTGCCGCATATGCTAATGTT
ACAACAGCCTTTCCATTTTGGCCTGGTCCATTTATGCCTTACCAGTTCCAGATTTTACCCTCTACTGGTAGCT
TTCCTCCAAATCCTGAACATTTCTTGCACCACAAATGATACACAGGCTACAAGATGAACTAGGACTGCACCA
CGAACGAGCCCTCTTCAGTAGTTCCTCCTCTTGGGAAGACTGCACGAGAGATGGCAGCATCTTTGTATGATG
GAACAGGAGCTAATGGTAATTTCTTGTATGGAATAATTCGTTCA**AGTCTTGAGATGGGTTTGCC**ATCATCTCA
CCCAAAGAAGGCAGTACAGGTGTGGGAAATAGCGGAAAAATGTCTAACAAAGCACTCATTGACCAGCTTTGTC
GTAACAGTTGTGCTACTCCTCTGCCCAAAGCTCCGACACCTTTTAGTATGAAAGATTGCAGTACTAGTGAAAT
AGCTCAGATGAAGAACCTGTAAAGCAAGAAAGTGTATCCCGAACAGCCCTTAGAATCTGCTTCTCCTGCAG
AGCCTGTAGTTTGTCTATCAAATTCATCCAGTGGCCTGCCAGAACATGACTGTAGAATAGATAGTGAACATGTT
ACTTCAGTTCCAGATGAAAAACATAGCAAGGACAGTGTTTTGCCACTGTCAGGGGTGCTGTGTGTCATCAA
CTGAAGATAGTTCTGAATTATTTACTAAAGATTCAAGTGAATCCATAAAGAACATGTTCATAATCCAAGTAA
GAAAGTGCCTGTGATAGTAAACTGTAAAAATACTTGGTGATATTGAAGCTGCCAAAGACTTACTCCCTAAAG
AAGAAGGCAACGAGGATTCAGAACAACTGAGGATACCAAATCAATGACGGTCAACTCATAG

Supplementary Figure S1: *SgE93* nucleotide sequence with primer sets indicated. Complete coding sequence of *S. gregaria E93* mRNA as found on NCBI (accession number LC215000.1) (Sugahara et al., 2017) with localisation of the different primers that were selected for production of the dsRNA construct or for performing qRT-PCR assays. Sequence regions highlighted in yellow indicate the position of the primer set that was used for generating the *dsSgE93* construct. Sequence regions highlighted in purple indicate the primer set used for qRT-PCR.

MAECSYARCVQERRAIKKELQRWTKNMVFVVGLELVAEELMGRRKWKHYQESVLRSPQADIEPSSSDWQVEDK
CCFCDGGMFLRPATSPQSDSCSSNSHSSFHETQSTVAADSSPPRLAMTTLEPVTSLAASLAAPTYSPPGAQHSA
MATTPPAGLSLYPTVARHRTAGLFPWPYLSPTAPAPNVRSEKTEPETPPVVVSLPASTPLSVPSAAASEQ**PLDLSA**
KAKEEASAPSTSPVPQPLGLATLSLSKQIFKAKPRMSAVAGRRTY**TEELQAALRDIQSGKLGTRRAAVIYGIPRST**
LRNKVYKLAMERERDAHLVVPTVEERDLGAEDEKEVERALSRLSSVDDLLRLSVLDGDAL**LRVLL**EEVSAGHNREA
SESASSPVFPQSELWHGLEQSAALGPYISQLLIAGSAQDPCHAESGGTLPKFTSPLLPELVRRMMAEEQQQIKKLG
SANNLEANTNITLNGASGDEIQKLGDSDNSAGPPRVPSASSQTDVPKATTKVGEADESSDTDATASTRVATPPNVI
LRIPSFKPTTKNGVATPGSGSLPSSEIPFQTVGVPAFPQGESSHVLNSSSNNSNESCSPASNLVGKGIGVSLRDVIK
SISQKFQPTDLPMSQKLLLSADEQPPFKRGRFTPLNTNITSCTIKHNNNNNTNVDDKNKTSSAGGKTSNSTG**KGT**
RPKRGRKYRNYDRDSLVEAVRAVQRGEMSVHRAGSYYGVP**HSTLEYKVKERHLMRPRK**REPQQQSDDEVKRKDE
SGTSIPRSTATTSSALANDKLKTLPKPKTAFNSPAPLPAGPNGLKIPPLFDPSLSLAAYANVTAFPFWPGPFHALPV
PDFTTPTGSFPPNPEHFFAPQMIHRLQDETRTAPRTSPSSVVPPLGKTAREMAASLYDGTGANGNFLDGIIRSSLEM
GLPSSSPKEGSTGVGNSGKMSNKALIDQLCRNSCVTPLPKAPTFSMKDCSTSVNSSDEEPVKQESVIPEQPLESASP
AEPVVCLSNSSSGLPEHDCRIDSEHVTSVPDEKHSKDSVPLSGVHCVSSTEDSSELFKDSSEFHKEHVHNPTESAC
DSKTVKILGDIEAAKDLLPKEEGNEDSEQTEDTKSMTVNS

Supplementary Figure S2: SgE93 amino acid sequence with protein domains indicated. The amino acid sequence of *S. gregaria* E93 as found on NCBI (accession number BBA65756.1) with localisation of the different protein domains. The amino acid sequence regions highlighted in yellow and green indicate the CtBP-interaction motif and NR-box, respectively. The amino acid sequences indicated in bold represent the two HTH-DNA binding motifs, RHF1 (bold and underlined) and RHF2 (bold).

```

SgE93  MAECSYARCVQERRAIKKELQRWTKNMVFVGLERVAEELMGRRKWKHYQESVLRSR-----
BgE93  -----MGRRRWKQYQDSVLRSP-----
DmE93  -----MHISSEISLERVAEECGRRWQWKHYQDKLTCSHLNIEEQQPIAIGSEDEPSQYNH
Bm2E93 -----MYWYPRDRKRNRGLERVAEELMGRRKWKLYQDALIPKR-NEQDDSDSDMPSTDPPPALKIK
BmE93  -----MGRRKWKLYQDALIPKR-NEQDDSDSDMPSTDPPPALKIK
LdE93  -----MGRRKWKLYQESMSRSYLQPLNNNIKRSNPPEEDLDEYHH

SgE93  -----PQADIEPSSSDWQVEDKCCFC-DGMFM
BgE93  -----LRVDVEPRIQDWEPQEKCCMC-DGKPF
DmE93  SSKEISQSNPNHCKTENHRLEQQHNGSQLLEEDSENNQTSHTDSSRTPTPGATSTSPPPPEPIDWRPSAKNFCVNGRLL
Bm2E93 TIEE-----INAPEDERPRVESDGNQESKTSRPETILESLIKRPATQPKVEVLEPADWKPPDKCYFCVDGEP-
BmE93  TIEE-----INAPEDERPRVESDGNQESKTSRPETILESLIKRPATQPKVEVLEPADWKPPDKCYFCVDGEP-
LdE93  HHKDYKQN-----LHPHQADSEGNEPKTGGKEEEARGEGGGGDRAIEGHQKEKVD-RLKDWTPQDKCYFCVDGKLD

SgE93  LRPAT---SPQSDSCSSSNHSSF-----HETQSTVAADSSPPRLAMTTLEP-----VTSLAAS
BgE93  FDSAT---SPAASDSGSTGTSSSL-----RETPPHEAAP-----AMTTLOS-----VTSMAAS
DmE93  TVNAQGKLVAESAATATSSSTSNSHI-----HQHDSDSNSSASLPHHISSSSSNNSSSGNRARHIAAA
Bm2E93 --RATAEAAQPVGATSPASESDSSSV-----SGTNSPAAAPPLLQHLQLQAQN-----PQTIAQF
BmE93  --RATAEAAQPVGATSPASESDSSSV-----SGTNSPAAAPPLLQHLQLQAQN-----PQTIAQF
LdE93  SEHTTHGVLSRPPSDTSDSHSDSEVPASILTPTSRGLNNNNHHHHRNVQHPVTPSSAMTTTIEN-----V-----S

SgE93  LAAVPTYSPGPAQGHSAMATTP--PAGLSLYPTVARHRTPAGLFPP-WYLSPTAP---APNVRSEGKTEPETPPVVVSL
BgE93  IAAVALSSSGTGTTTNPLLPSPQSAMPFYP-----PPPHFPP-WYLSPPHSLPGRPVLADAKQDPSATPPAVVHL
DmE93  SARATPAAATPANSLELYKLLTQRAAKMTSMDMAAQLAQFSLLAD-FNLINSLASQQQQQQQQQIASAVTPTTSEVSAA
Bm2E93 QQMIAALT-ALGTGLV-----PPPLTQA-WMMQRFAQQRHQADRLSESDKAAAPSSP-----
BmE93  QQMIAALT-ALGTGLV-----PPPLTQA-WMMQRFAQQRHQADRLSESDKAAAPSSP-----
LdE93  MAALAAAALSGGASPGVTPAAP---HLPFYN-----PASVLTHNWYLANVVRFQNAANDAAVDKAS-----

SgE93  PASTPLSVPSAAASEQPLDLSAKAKEAS-APSTSPVPQPLGLATLSLDSKQI---FKAKPRMSAVAGRRTYTEELQA
BgE93  PASS-----SASEQPLDLSAKTAA---VEIPSPAPPDPSLKVPSIDNKHI---FKAKPRMSTVAGRRTYTEELQA
DmE93  AISPALKDTPSPSVDAPLDLSK-----PSPNSSISGDVKSVRACAT-----PTPSGRRAYSEEDLSR
Bm2E93 -----SPVEQPLDLSAKSTSSSTSGTPPPDPKFLDSRLRRTALDGA-----SNSTGRRTYTEDELQS
BmE93  -----SPVEQPLDLSAKSTSSSTSGTPPPDPKFLDSRLRRTALDGA-----SNSTGRRTYTEDELQS
LdE93  -----GAGEQPLDLSKGSNNNP-PATDRKVPLAINVRLPTLDTKHIFNSDFRAKPRMSAVAGRRTYTEDELQA

SgE93  ALRDIQSGKLGTRRAAVIYGIPRSTLRNKVYKLAMERERDAHLVVPTV-----EERDLSGAEDEKEVERALSRPL
BgE93  ALRDIQSGKLGTRRAAVIYGIPRSTLRNKVYKLALEKERDSHLVAPAVPKVEE---DEKELSGAEEREVEKALRKPL
DmE93  ALQDVVANKLDARKSASQHEQRSTLDNRLFKMKHHDQFQDHDG-----DELEDSDNDAEAEVDSNASTTPV
Bm2E93 ALRDIQSGRLGTRRAAVLYGIPRSTLRNKVNKE-----
BmE93  ALRDIQSGRLGTRRAAVLYGIPRSTLRNKVNKE-----
LdE93  ALRDIQSGKLGTRRAAVIYGIPRSTLRNKVYKLALERERESHINSSAPLKLEEEEAMDDDKELSGAEEREVEKALQAPL

SgE93  LSVD---DLLRLSVLDGDALRVLLEEVSAGHNREASESASSPVFPQSSSELWHGLEQSALGPYI-SQLLIA--GSAQDPC
BgE93  LSME---DLVRFVFKDAN-----YPDA---WGGLEHSALGPYV-AELLAAQKNPASTPG
DmE93  YPAEFARAQLRKLSHLSEHNGSDLGEDVD---RGSPKMGRHPACGNASANQGAPPSIPLDANVLLHTLMLAAGIGAMPK
Bm2E93 -----GLVADNH
BmE93  -----GLVADNH
LdE93  LSMA---DLYRFGS-----REQPPDALKTLLQRG--KEGMNPSEMGPYI-KNLIMASQNILANQK

SgE93  H--AESGGTLPKFTSPLLPELVRRMMAEEQQQIKKLGSANNLEANTNITLNGASGDEIQKLGDSDNSAGPPRVPSASSQ
BgE93  DKSPTSGDFLPKFPTPLLPEFVRRMMAEDKLQSFHP-----NGAHADERE-----PER-----
DmE93  ---LDETQTVGDFIKGLLVANSGGIMNEGLNLLLSA-----SQENSNGNASLLLQQQHHQHHQHHQHHQ
Bm2E93 D--SD-----PDS-----
BmE93  D--SD-----PDS-----
LdE93  S--PEG-----TPMIPELMKMIAEEQLLKQHQ-----QYNNGDNERLLRPS-----PSN-----

SgE93  TDPVKATTKVGEADESSDTDATASTSRVATPP-NVILRIPSFKPT-TKNGV---ATPGSGSLPSSEIPQTVGVPAFP
BgE93  -----PLRDQESPDSPPGMATPPSNVILKIPSFKPT-SKNGV---ASSSGSTEPPPPLP-----PPPP
DmE93  QQHVAAYRHLPKSETPTNSSLDPNDASEDP--ILKIPSFKVSGPASS---SLSPGGLVGHHHPLNNNNSLSIS
Bm2E93 -----DQDRAES-----PP-SVILKIPTFPPDDKSPS---PATPVTTPTITPLTPLISQPPPSLN

```

```

BmE93  -----DQDRAES-----PP-SVILKIPTFPFPDDKSPS-----PATPVTTPTITPLTPLISQ-----
LdE93  ----SVITKIERAKSESDMETEDS-----PS-NVILKIPSFPRPTSSKNGCDIFRNPEATGSMISPPVT-----

SgE93  GQE--SSHVLNSSSNNSNESCSPPASNLVGKG-----IGVSLRDVIAKSIQKFFQPTDLPMSSQKLLLSAD----
BgE93  PES--SSQQL-----SDSCSPVPVPSLVGKG-----IGVSLRDVIAKSIQKFFQHS-ELSPKLGMPVE----
DmE93  NNSNHSSNSHRNGSNRSPHSASPLAAVAQGGYSAGNSLLTSSSSSIQKMMASNIQRQINEQSGQEQLRNGNVSDCSSN
Bm2E93 PPS--NLLLSP-----SVFADPPAGSQH-----IFTSLNDVIAKSIQKFFQ--PLDRTHQ-----
BmE93  -----PPAGSQH-----IFTSLNDVIAKSIQKFFQ--PLDRTHQ-----
LdE93  -----SESGSPPIPNKGL-----IHKDVKDVIQSIQKFFQ--SLEPRRPI-----

SgE93  ----EQPPFKR-----GRFTPLNTNITSCT-IKHNNNNNTNVDDKNK-----TSSAGGKTSSNSTG
BgE93  ----TEPPFKR-----GRFTPLVAGASATSVIKHNNNNNSQADDRNAQKILPQVQSKPTGTSSGASSQSSSSGG
DmE93  NGGSSSLGYKKPSISVAKIIGGTDTSRFGASPNLLS-QQHSAHHLTHQQQQ--QLSAQEALG
Bm2E93 ----ADLSFMR-----APDARHVSVIK-SQSDNQRYAMPSSNK-----VPTNNGQAAAGG
BmE93  ----ADLSFMR-----APDARHVSVIK-SQSDNQRYAMPSSNK-----VPTNNGQAAAGG
LdE93  ----IEVDFKR-----GRFTPLGAGISVIK-TQHEMNRQYQPPPKPQ-----QHLNDTTTTTGG

SgE93  KGTTRPKRGKYRNYDRDSLVEAVRAVQRGEMSVHRAGSYYGVPHSTLEYKVKERHLMRPRKREPQQQSDVEVRKDESGTS
BgE93  KGTTRPKRGKYRNYDRDSLIEAVRAVQRGEMSVHRAGSHFGVPHSTLEYKVKERHLMRPRKREPQPPMEDAKKKEE----
DmE93  KGTTRPKRGKYRNYDRDSLVEAVKAVQRGEMSVHRAGSYYGVPHSTLEYKVKERHLMRPRKREPQPPDLVGLT-----
Bm2E93 KGTTRPKRGKYRNYDRDSLVEAVKAVQRGEMSVHRAGSYYGVPHSTLEYKVKERHLMRPRKREPQPPQDTKPQ-----
BmE93  KGTTRPKRGKYRNYDRDSLVEAVKAVQRGEMSVHRAGSYYGVPHSTLEYKVKERHLMRPRKREPQPPQDTKPQ-----
LdE93  KGTTRPKRGKYRNYDRDSLVEAVRAVQRGEMSVHRAGSYYGVPHSTLEYKVKERHLMRPRKRDPRPNPVDEKLATLK----

SgE93  IPRSTATTSSALANDKLKTLPKP-PK-----TAFN-SPAPLPGAPNGLKIPPLFDPS-LSLAAYANVTTAF
BgE93  -----TRHPAATLEKSKIPPSTPK-----TPYTSSSAIPSAFNGLKLPMPFDPANVPMPYAT-APPF
DmE93  -----GPANKLQLDKLGKAGPHGGSKLSNALKNQNNQAAAAAATPNGLK-PLFEAGPQALS-----FQPN
Bm2E93 -----PPKPLPP-----PKP-----PGKP-----FSNGLNGPETPTY-PAGY
BmE93  -----PPKPLPP-----KP-----PGKP-----FSN-----
LdE93  -----QNDIRLAQDKMKPMKP-PQ-----QKYP-----PTSPNGMKL-PIFEPGMTPLAGYN-APPF

SgE93  PFW-PGP-FHALPVPDFTTPTGTFPPNPEHFFAPQMIHRLQDETRTAPRTSPSSVVPPLGKTAREMAASLYDGTGANGNF
BgE93  PFWPPNP-FHSLPLP---PAG-FSPTQD-FFASQMMQRLGGGASSPAALSTSSRSPPLGKSAREMAESLYDGTGANGSF
DmE93  MFWPQTNAATNAYGLD-----FNRITEAMRNPOASNHH-----GLMKSQDMVENVY-----
Bm2E93 PFW-ASPGFA-----PPPTPDLYASHMMRRLREEAPP-----ANGSF
BmE93
LdE93  PFWPHHGFAGHLPLDYARNPTSPFPPSPDFFASQMMQKLQEESSRTMSAVPS---PALAKNARQMAESLLEGPGANGSF

SgE93  LDGIIRSSLEM---GLPSSSPKEGSTGVG-NSGKMSNKALIDQLCRNSCVTLPKAPTFFSMKDCSTSVNSSDEEPVKQ
BgE93  LDGIIRSSLEM---GLPPGVPKEAE-----NMSNKALLDQLCRNSRLTLPRAAVTL---IDGGASSSDEDSIKR
DmE93  -DGIIRKTLQASEGNGSAAAGNSNGSNGNGHGHGHGHGHALLDQLLVKKTPLPFTNHRN---NDYAATCSSASGESVKR
Bm2E93 LEGIIRSSLER-----PGAALMQLASE-----
BmE93
LdE93  LDGIIRSSLES---GVPVSEEKSSNEKNLAPENMSNKALLDQLCRNSRLTPLSKPAA-----TTDGNSSGDESYAKG

SgE93  ES---VIPEQPLESASPAEPVVCLSNSSSGLPEHDCRIDSEHVTVPDEKHSKDSVLPISGVHCVSSTEDSSELEFTKDS
BgE93  SSSRNAFTCSRATESDASTSAVDLSPSSNGSTVERK---HI---DEEGEETMSP-----SATKPEDAEFSQET
DmE93  SG-----SPMGNYADIKRERLSADSGGSSDEEHSASHINNNNSDLAHNKNKSGGGGGGGNGQTNGNGRSSRMTSRDD
Bm2E93 -----SPSLVRRLA
BmE93
LdE93  LS-----PLNFATGASHEDNDDSSRYEKDV-----SDVNAIELS

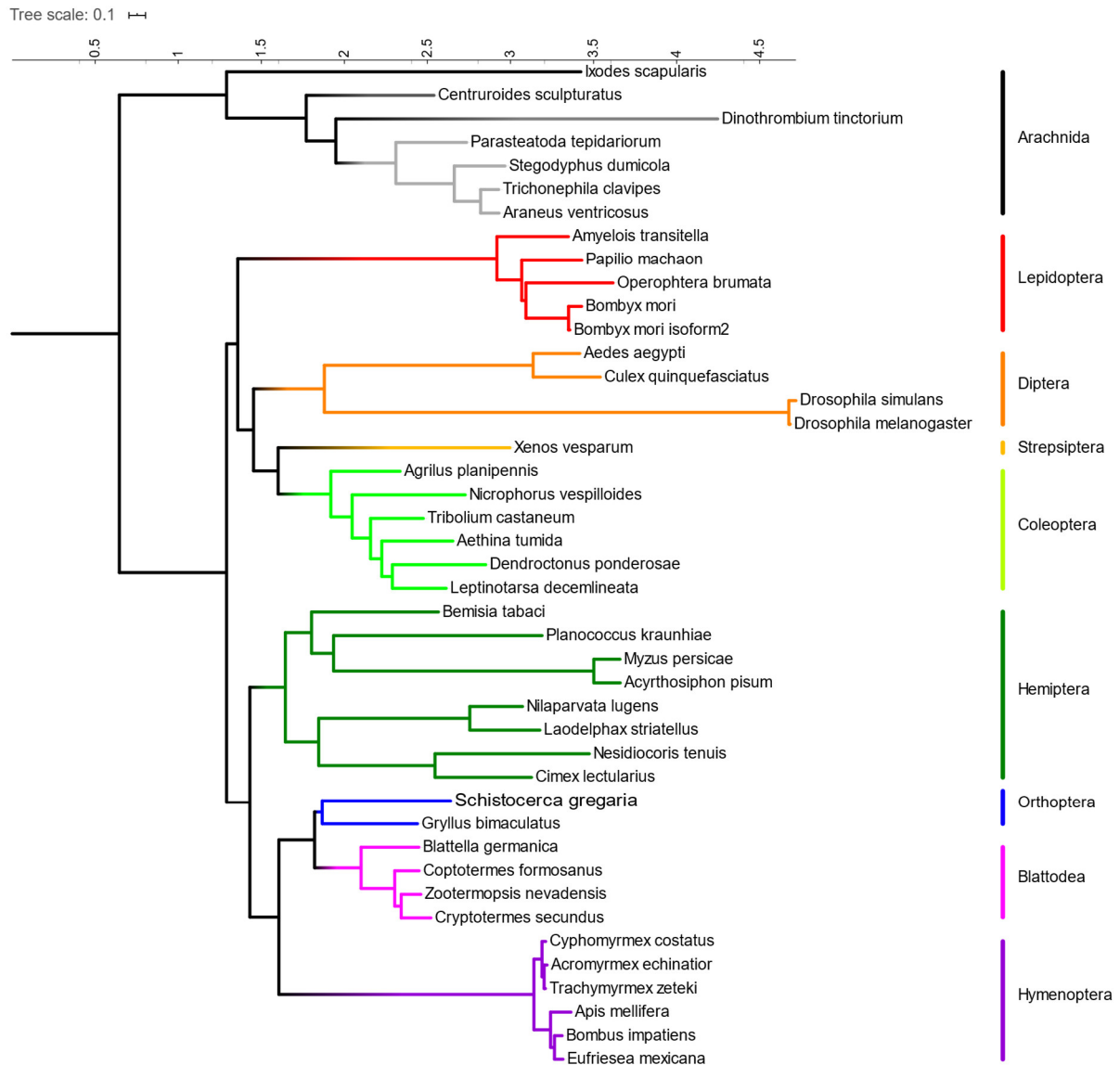
SgE93  SEFHKEHVHNP-----EESACDSKTVKILGDIKADLLP-----KEEG-----NEDSEQEDTKSMT
BgE93  NGISNDNDNNATEDTDGNDNDNEESACDSKNITKRVEDAAKDDKQ-----DDAP-----EEEIEENEETKS-K
DmE93  SETDASSLKSGE-----SGGQONHKMMDLNGGSSSSSHIKCESEATGHHSPGHHTTSILHEKLAQIKAEQVDQ
Bm2E93 ADVVEEPAARRPR-----LDSSDHQLAAEMREAVQRLRAD-----KLRP-----RNGTPAPSPPPAPP
BmE93 -----KLRP-----RNGTPAPSPPPAPP
LdE93  NDSNASSERKAT-----DETERKQPRIYLNQDLAKPENLKPEMLVRFREALD-----HNLNESSASENDG

SgE93  VNS-
BgE93  INS-
DmE93  ADQL
Bm2E93  DRA-
BmE93  DRA-
LdE93  PQD

```

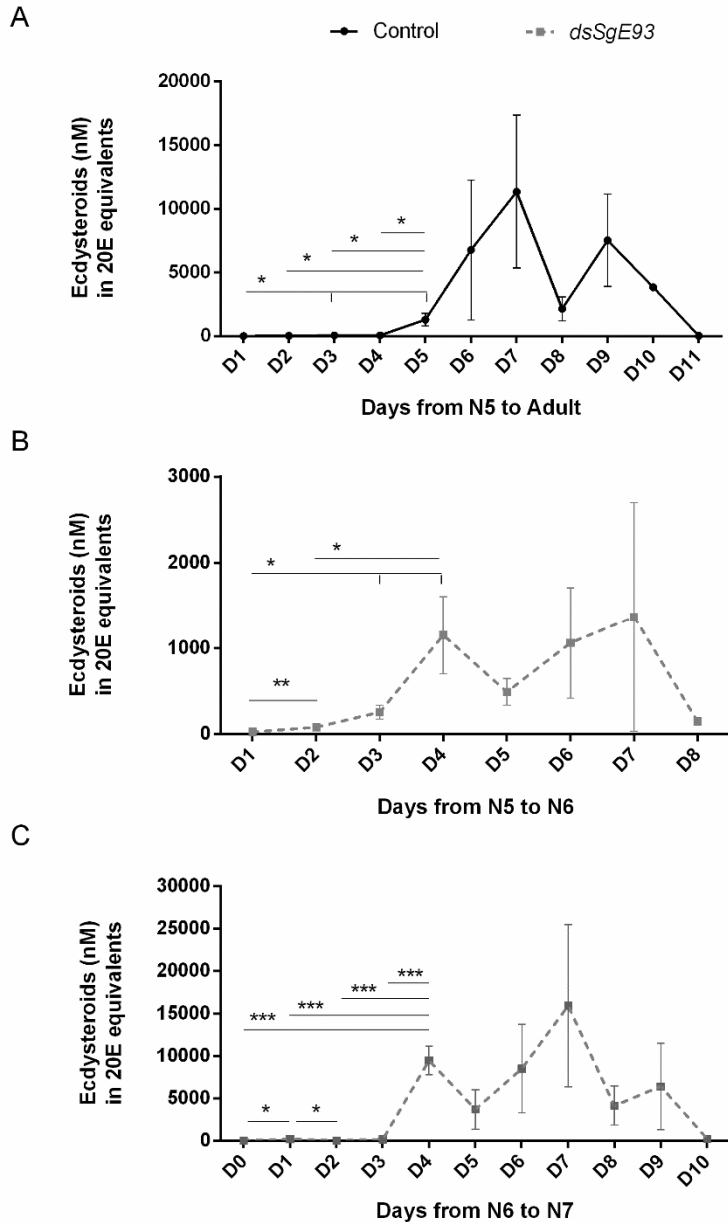
Supplementary Figure S3: Multiple sequence alignment of SgE93 with other E93 proteins. The multiple sequence alignment of SgE93 (BBA65756.1) with E93 proteins from the German cockroach, *Blattella germanica* (BgE93, CCM97102.1), the fruit fly, *Drosophila melanogaster* (DmE93, NP_652002.2), the silkworm, *Bombyx mori* (BmE93 and Bm2E93 sequences were obtained from

AIL29268.1 and the ORF of ICPK01035599.1, respectively) and the Colorado potato beetle, *Leptinotarsa decemlineata* (LdE93, AQN67828.1). The sequences were aligned with MUSCLE and colored by 70% consensus using MView. Several domain structures for E93 are shown: the two HTH-DNA binding motifs, RHF1 (highlighted in yellow) and RHF2 (highlighted in blue), the CtBP-interaction motif (CtBP-im, highlighted in pink) and the NR-boxes (highlighted in grey).

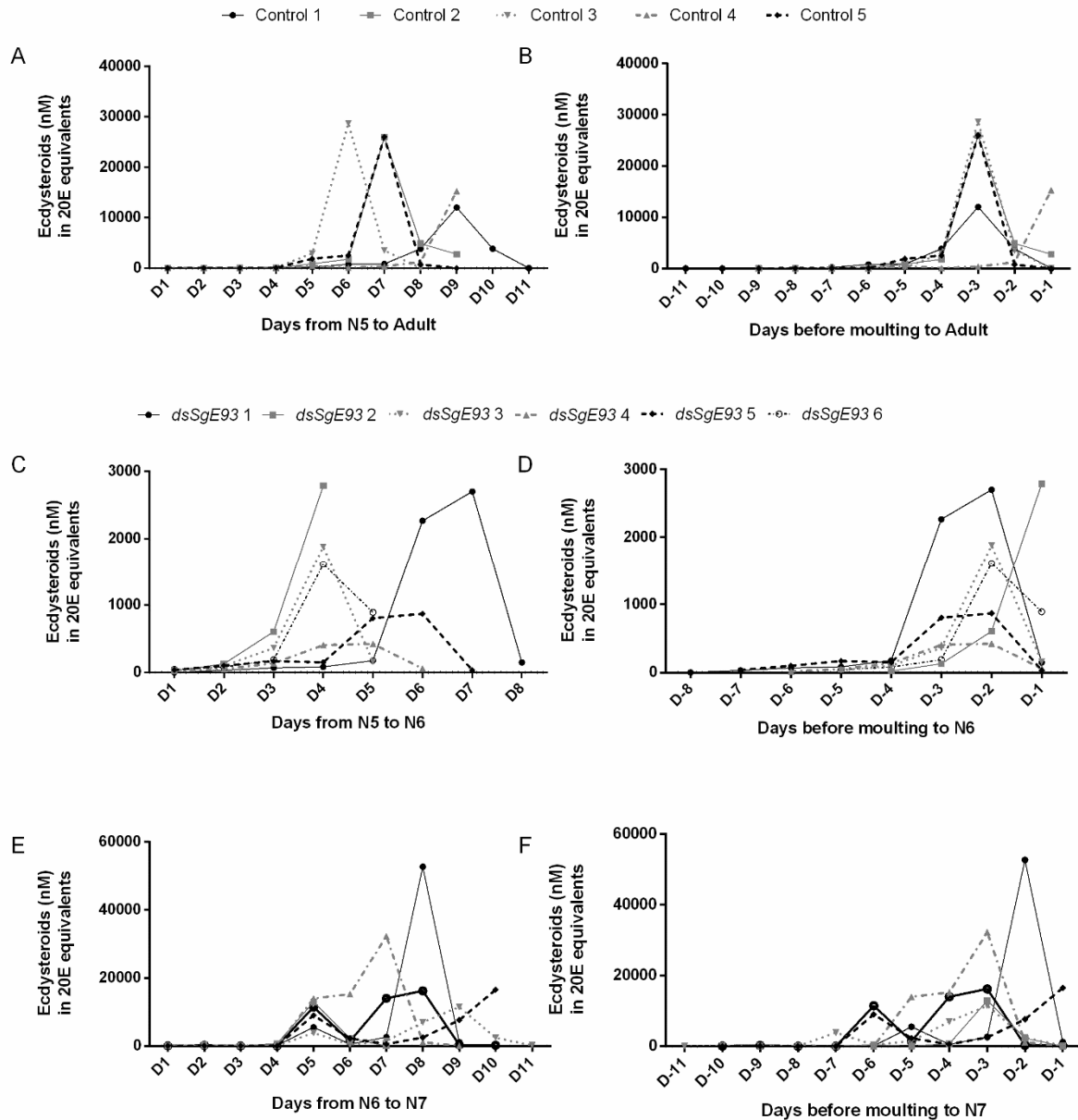


Supplementary Figure S4: phylogenetic analysis of E93 orthologs from insect and arachnid species.

E93 sequences listed in Supplementary Table S1 were aligned with MUSCLE and used for a maximum likelihood analysis using IQ-tree. The resulting tree was rooted using the Arachnida class branch as the outgroup. The branches are colour coded according to the insect order. Scale values indicate evolutionary distance. Tree rendered with iTol.



Supplementary Figure S5. Temporal ecdysteroid profiles in haemolymph of *dsSgE93*- and *dsGFP*-injected desert locust nymphs. Ecdysteroid levels (20E equivalents in nM) were measured in haemolymph samples that were collected daily from *dsGFP*-injected (control) fifth nymphal (N5) locusts (A), as well as *dsSgE93*-injected (*SgE93* knockdown) fifth nymphal (N5) (B) and sixth nymphal (N6) (C) locusts. Data are shown as the number of days after the previous (N4-to-N5) moult. Each data point in the figure represents mean $\pm$ S.E.M of 5 control (*dsGFP*) or 6 experimental (*dsSgE93*) locusts. Statistically significant differences between the measurements (starting on D0/1 until the following ecdysteroid peak) were found via a t-test (with or without two-sided Welch's correction) and are indicated by (an) asterisk(s) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



Supplementary Figure S6: Temporal ecdysteroid profiles in haemolymph of control (*dsGFP*) and *SgE93* knockdown (*dsSgE93*) nymphs. Ecdysteroid levels (20E equivalents in nM) were measured in the haemolymph of *dsGFP*- (control) and *dsSgE93*- (knockdown) injected locusts in the fifth nymphal stage (A - D) and of *dsSgE93*- (knockdown) injected locusts in the sixth nymphal stage (E + F). Each data point represents a measurement and each line represents an individual locust. In total, haemolymph samples from 5 control (*dsGFP*) and 6 experimental (*dsSgE93*) locusts were analysed.

Supplementary Table S1: Species and accession numbers of sequences used for constructing the phylogenetic tree shown in Suppl. Fig. S4.

Species:	Accession number:
<i>Acromyrmex echinator</i>	EGI58724.1
<i>Acyrtosiphon pisum</i>	XP 008189864.1
<i>Aedes aegypti</i>	EAT44017.1
<i>Aethina tumida</i>	XP 019872192.1
<i>Agrilus planipennis</i>	XP 018321151.1
<i>Amyelois transitella</i>	XP 013188059.1
<i>Apis mellifera</i>	BAB64310.1
<i>Araneus ventricosus</i>	GBL83064.1
<i>Bemisia tabaci</i>	XP 018900968.1
<i>Blattella germanica</i>	CCM97102.1
<i>Bombus impatiens</i>	XP 003490553.1
<i>Bombyx mori</i>	AIL29268.1
<i>Bombyx mori isoform 2</i>	ICPK01035599.1 derived
<i>Centruroides sculpturatus</i>	XP 023227973.1
<i>Cimex lectularius</i>	XP 014260111.1
<i>Coptotermes formosanus</i>	GFG28607.1
<i>Cryptotermes secundus</i>	XP 023704791.1
<i>Culex quinquefasciatus</i>	XP 001863525.1
<i>Cyphomyrmex costatus</i>	KYN00623.1
<i>Dendroctonus ponderosae</i>	XP 019771885.1
<i>Dinothrombium tinctorium</i>	RWS15376.1
<i>Drosophila melanogaster</i>	NP 652002.2
<i>Drosophila simulans</i>	EDX13858.1
<i>Eufriesea mexicana</i>	XP 017758118.1
<i>Gryllus bimaculatus (partial)</i>	BBJ70113.1
<i>Ixodes scapularis</i>	EEC08637.1
<i>Laodelphax striatellus</i>	RZF34802.1
<i>Leptinotarsa decemlineata</i>	AQN67828.1
<i>Myzus persicae</i>	XP 022167663.1
<i>Nesidiocoris tenuis</i>	CAA9993019.1
<i>Nicrophorus vespilloides</i>	XP 017770390.1
<i>Nilaparvata lugens</i>	AP013600.1
<i>Operophtera brumata</i>	KOB75508.1
<i>Papilio machaon</i>	KPJ20458.1
<i>Parasteatoda tepidariorum</i>	XP_015926289.1
<i>Planococcus kraunhiae</i>	BBD13408.1
<i>Schistocerca gregaria</i>	BBA65756.1
<i>Stegodyphus dumicola</i>	XP 035205796.1
<i>Trachymyrmex zeteki</i>	KYQ50221.1
<i>Tribolium castaneum</i>	KYB25179.1
<i>Trichonephila clavipes</i>	PRD31503.1
<i>Xenos vesparum</i>	AYD60146.1
<i>Zootermopsis nevadensis</i>	KDR22086.1

Supplementary Table S2: Oligonucleotide sequences of primers used for qRT-PCR.

Reference/ target genes	Forward primer	Reverse primer
<i>SgAct</i>	5'- AATTACCATTGGTAACGAGCGATT -3'	5'- TGCTTCATACCCAGGAATGA -3'
<i>SgEF1α</i>	5'- GATGCTCCAGGCCACAGAGA -3'	5'- TGCACAGTCGGCCTGTGAT -3'
<i>SgE93</i>	5'- CGCAAGCAGACATAGAACCC -3'	5'- TGGTCTCAGGAACATCCCAC -3'
<i>SgMet</i>	5'- GGTGCCTGAAGAGGAAGAAA -3'	5'- ATGGAGGTGATGAAGGAGAAAAG -3'
<i>SgKr-h1</i>	5'- CTCGAAGACGTTTCATCCAGAG -3'	5'- TGCTTGGAGCAGGTGAAG -3'
<i>SgCYP15A1</i>	5'- AAAGCAACTTCATCATTACAGATG -3'	5'- CAGAGCCAGCCATGAACAAA -3'
<i>SgJHAMT</i>	5'- CGGAGCAAAGGCAAGCA -3'	5'- CCACTTCACCGCCTGGTTT -3'
<i>SgSpo</i>	5'- CAACATCTTACCAGCTACATGTG -3'	5'- GGGTCGTCGTAGTCGAAGGA -3'
<i>SgPhm</i>	5'- CGCAGAGCCCGGACAAC -3'	5'- CGAACATGTCGGCCATGA -3'
<i>SgSad</i>	5'- ATCGTGGCCGAGATTACGAA -3'	5'- AGCACCATCTCCGATCCT -3'
<i>SgDib</i>	5'- CCCAGGCTGCTATCGAGACT -3'	5'- CGACGACCGGCTATGTAGTT -3'
<i>SgEcR</i>	5'- AAGGTTGATAATGCGGAATATGC -3'	5'- GTGATGGGCGCTCTGAAAAT -3'
<i>SgRXR</i>	5'- AATGCCTCGCTATGGGAATG -3'	5'- TCCTTTGTTGCTGCCTTTC -3'

Abbreviations: *Sg* = *Schistocerca gregaria*; *Act* = β -actin; *EF1α* = *Elongation factor 1α*; *Met* = *Methoprene-tolerant*; *Kr-h1* = *Krüppel-homolog 1*; *CYP15A1* = *Cytochrome P450 enzyme 15A1 (methyl farnesoate epoxidase)*; *JHAMT* = *Juvenile hormone acid methyltransferase*; *Spo* = *Spook*; *Phm* = *Phantom*; *Sad* = *Shadow*; *Dib* = *Disembodied*; *EcR* = *Ecdysone receptor*; *RXR* = *Retinoid X receptor*.

Supplementary Table S3: Oligonucleotide sequences of primers used for dsRNA synthesis.

Nucleotides indicated in 'bold' represent the T7 promoter sequences used for preparing the dsRNA constructs.

RNAi constructs	F-primer	R-primer
<i>dsSgE93</i>	5'- TAATACGACTCACTATAGGGGAGA GCCCAAGCAACAACAGTCTG -3'	5'- TAATACGACTCACTATAGGGGAGA GGCAAGCCCATCTCAAGACT -3'
<i>dsGFP</i>	5'- TAATACGACTCACTATAGGGGAGA AAGGTGATGCTACATACGGAA -3'	5'- TAATACGACTCACTATAGGGGAGA ATCCCAGCAGCAGTTACAAAC -3'

Abbreviations: *Sg* = *Schistocerca gregaria*; *GFP* = *Green fluorescent protein*.