SAPIENZA
UNIVERSITÀ EDITRICE

E-ISSN 2284-4880
ISSN 0429-288X

Fragmenta entomologica, 54 (2): 273–278 (2022)

Research article
Submitted: December 20th, 2021 – *Accepted*: July 8th, 2022 – *Published*: December 15th, 2022
DOI: 10.13133/2284-4880/705

Body pigmentation during embryogenesis first found in stoneflies: a case of *Megaperlodes niger* Yokoyama, Isobe & Yamamoto, 1990 (Insecta: Plecoptera, Perlodidae)

Shodo MTOW[1,*], Tadaaki TSUTSUMI[1]

[1] *Faculty of Symbiotic Systems Science, Fukushima University, Kanayagawa 1, Fukushima, Fukushima 960–1296, Japan* – impulse610@gmail.com (SM); thrips-tsutsumi@sss.fukushima-u.ac.jp (TT); ORCID: https://orcid.org/0000-0003-0092-5823 (SM)
* Corresponding author

Abstract
We examined and described the later stages of embryonic development and first instar nymphs of the stonefly *Megaperlodes niger* Yokoyama, Isobe & Yamamoto, 1990 to document its body pigmentation during embryogenesis. Pigmentation commences at the periphery of egg tooth when the definitive dorsal closure is almost completed. Full-grown embryos have their heads pigmented dark-red, the first thoracic segment yellowish in colour, and the posterolateral margin of second and third thoracic segments slightly reddish. The colouration of first instar nymphs is almost the same as full-grown embryos, and the lateral margin of the first two abdominal segments is pigmented red. Our finding that body pigmentation occurring at *M. niger* embryogenesis is the first known report of plecopteran embryos and hatchlings, contributing to further understanding of the nymphal biology of this species.

Keywords: Arctoperlaria, Systellognatha, pigmentation, embryonic development, first instar nymph

Introduction

Plecoptera is a hemimetabolous, polyneopteran order with approximately 3,700 described species distributed globally, except for Antarctica (Fochetti & Tierno de Figueroa 2008; DeWalt & Ower 2019). This order comprises two suborders, Antarctoperlaria and Arctoperlaria, including 17 extant families (e.g., Zwick 2000; DeWalt & Ower 2019; South et al. 2021b). The Antarctoperlaria is present only in the Southern Hemisphere, containing four families, whereas the Arctoperlaria, mostly inhabiting the Northern Hemisphere, is composed of two subgroups, Euholognatha and Systellognatha. Each subgroup contains six and seven families, respectively; recently, a new family, Kathroperlidae, comprising only one genus, *Kathroperla* Banks, has been established as a sister group of systellognathan Perloidea (= Perlidae + Chloroperlidae + Perlodidae) based on morphological and phylogenomic studies (South et al. 2021b).

Perlodidae is a systellognathan family present mainly in the Holarctic region, containing 340 described species, and is divided into two subfamilies, Isoperlinae and Perlodinae (e.g., Zwick 2000; DeWalt & Ower 2019). Recent molecular phylogenetic analyses, including phylogenomic studies, support the monophyly of Perlodidae (e.g., Terry 2004; Letsch et al. 2021; South et al. 2021a), despite the lack of defining morphological apomorphies (Zwick 2000). However, the monophyly of each subfamily is supported by morphological evidence based on gills and terminal abdominal structures (Zwick 2000), whereas it is sometimes obscured according to molecular phylogenetical studies (e.g., Terry 2004; Letsch et al. 2021; South et al. 2021a).

Megaperlodes Yokoyama, Isobe & Yamamoto, 1990, belonging to Perlodinae, is a small genus consisting of only two species: *Megaperlodes niger* Yokoyama, Isobe & Yamamoto, 1990, which is endemic to Japan, and *Megaperlodes tiunovi* Teslenko, 2015 distributed in Russian Far East and Korea (Yokoyama et al. 1990; Inada et al. 1998; Yoshinari 2001; Murányi & Hwang 2015; Teslenko 2015). For these two species, the morphology of adults, older nymphs, and egg structures have been described (Inada et al. 1998; Shimizu et al. 2005; Teslenko 2015); information on the biology of *M. niger* adults are also reported (Chino 2011). However, to date, we have no data concerning the embryology and earlier nymphal stages of these species.

Information on the embryonic development and first instar nymphs of Plecoptera can be a potential source of phylogenetic information (e.g., Harper 1979; Sephton & Hynes 1982; Mtow & Machida 2018; Mtow & Tsutsumi 2021). However, despite several detailed studies of embryology (e.g., Miller 1939; Kishimoto & Ando 1985; Mtow & Machida 2018) and morphology of the first instar nymphs (e.g., Harper 1979; Sephton & Hynes 1982; Mtow & Tsutsumi 2021), there is no report regarding body pigmentation of Plecoptera except for compound eyes: the body pigmentation begins in the second instar nymphs according to Khoo (1964).

In the present study, we examined and described the embryonic development and first instar nymphs of *M. niger* for the first time to document its body pigmentation.

Material and Methods

Female *Megaperlodes niger* was collected from Japan, Fukushima, Inawashiro, the Tatsusawa river; 780 m; around 37.35.24.000N, 140.13.10.003E; 24 May 2020 (Figs 1–2). The individual was kept at room temperature in a glass case containing tissue paper and fed on Okuwa Bari-bari-jelly, i.e., a commercial food for insects. Eggs deposited by the female and first instar nymphs were incubated in plastic cases filled with water at 12°C. Then, they were fixed with Karnovsky's fixative [2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M HCl-sodium cacodylate buffer (SCB), pH 7.2] for 24 h and stored in SCB at 4°C. The following measurements were taken from the fixed first instar nymphs: (1) body length (from the top of the head to the tip of the abdomen), (2) antennal length, (3) head width, (4) pronotum width, and (5) cercus length.

The fixed specimens and living eggs slide-mounted in distilled water were examined using an Olympus BX43 biological microscope and photographed using a Pentax K-70 camera. Then, the living eggs were soaked in Kao Kitchen Highter, i.e., a commercial bleach, for several seconds to remove the anchor plate and decolourise the brownish chorion before examination.

The specimens examined in the present study have been deposited in the collection of the Faculty of Symbiotic Systems Science, Fukushima University.

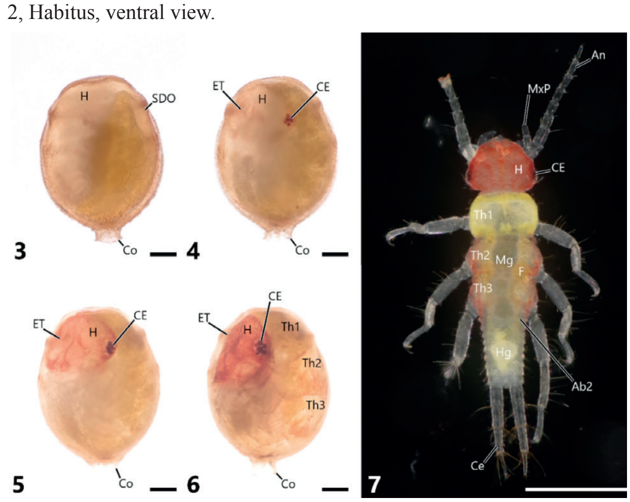
Results

Staging used in the description of embryonic development of *Megaperlodes niger* is based on Mtow & Machida's staging (2018, 2021) for the embryogenesis of the scopurid stonefly *Scopura montana* Maruyama, 1987, dividing it into 12 stages. In the present study, only the later stages, i.e., Stages 10–12, are mentioned.

Embryonic development. The egg period is approximately 330–360 days at 12°C. No data were obtained on the diapause period.

In Stage 10, which is the progression of the definitive dorsal closure, the secondary dorsal organ formed on the dorsal side of the head is recognisable, and the embryos are unpigmented (Fig. 3). In Stage 11, the definitive dorsal closure almost completes, the compound eyes are pigmented red, and a sclerotised, conical-shaped egg tooth forms on the frons (Fig. 4). Body pigmentation commences at early Stage 11, and the periphery of the egg

Figs 1-2 – Female adults of *Megaperlodes niger*. 1, Habitus, dorsal view; 2, Habitus, ventral view.



Figs 3-7 – Embryonic development and the first instar nymph of *Megaperlodes niger*. 3–6, Embryonic development, Stage 10 (3), Early Stage 11 (4), Later Stage 11 (5), Stage 12 (6), lateral view, anterior at the top; 7, First instar nymph, habitus, dorsal view, anterior third of the left side of antenna artificially lacking. Abbreviations: Ab2, second abdominal segment; An, antenna; Ce, cercus; CE, compound eye; Co, collar; ET, egg tooth; F, fat body; H, head; Hg, hindgut; Mg, midgut; MxP, maxillary palp; SDO, secondary dorsal organ; Th1–3, first, second, and third thoracic segments. Scale bars: 100 µm (Figs 3–6); 500 µm (Fig. 7).

tooth is slightly pigmented (Fig. 4); then, in later Stage 11, the head becomes dark-red (Fig. 5). Finally, in Stage 12, the embryos acquire the definitive form of the first instar nymphs, and head pigmentation further progresses (Fig. 6). The first thoracic segment is pigmented yellow but hardly recognisable due to the brownish chorion (Fig. 6). The posterolateral margin of the second and third thoracic segments is slightly reddish (Fig. 6).

First instar nymphs. Measurements of first instar nymphs are as follows (mean ± SD, µm, n = 5): body length: 1163.6 ± 26.2; antennal length: 564.4 ± 17.8; head width: 318.9 ± 11.6; pronotum width: 305.7 ± 13.1; cercal length: 409.2 ± 20.6.

The first instar nymphs have a slender body that is sparsely covered by fine setae, without gill and ocelli, and their colouration is almost the same as full-grown embryos (Fig. 7). The head is dark-red, prognathous, and subtriangular, being wider toward the posterior (Fig. 7). The antenna is unpigmented and composed of nine antennomeres, slightly shorter than half of the body length (Fig. 7). The unpigmented maxillary palp is recognisable from the dorsal view (Fig. 7). The first thoracic segment is brightly yellowish (Fig. 7). The posterolateral margin of the second and third thoracic segments is pigmented red (Fig. 7). Thoracic appendages are unpigmented (Fig. 7). Abdominal segments are unpigmented except the lateral margin of the first two segments, which was red (Fig. 7). Cerci are unpigmented and four-segmented, longer than one-third of body length, with a crown of long and short stout setae on the posterior margin of the first three segments; short stout setae on the apex of the fourth segment (Fig. 7).

Fat body, midgut, and hindgut are visible through the second- and third thoracic and abdominal targa (Fig. 7).

Discussion

Embryological studies on Plecoptera have been reported from ten arctoperlarian families, i.e., Euholognatha, Scopuridae (Mtow & Machida 2018), Taeniopterygidae (Khoo 1968b; Mtow & Machida 2018), Leuctridae (Mtow & Machida 2018), Capniidae (Kishimoto 1997; Mtow & Machida 2018), and Nemouridae (Kishimoto 1997; Mtow & Machida 2018), Systellognatha, Pteronarcyidae (Miller 1939), Peltoperlidae (Mtow & Machida 2018), Perlidae (Kishimoto 1997; Kishimoto & Ando 1985; Mtow & Machida 2018), Chloroperlidae (Kishimoto 1997; Mtow & Machida 2018), and Perlodidae (Khoo 1968a; Kishimoto 1997; Mtow & Machida 2018). Further, information on the first instar nymphs has been reported for 14 families, except for antarctoperlarian Diamphipnoidae, arctoperlarian Styloperlidae, and Kathroperlidae (e.g., Helson 1935; Miller 1939; Khoo 1964; Berthélemy 1979; Harper 1979; Sephton & Hynes 1982; Mtow & Tsutsumi 2021). However, no attention has been paid to the body pigmentation of embryos and first instar nymphs, except compound eyes, because their body was unpigmented or white in colour (Brinck 1949; Khoo 1964; Hynes 1976). In the present study, we found that the body pigmentation of *Megaperlodes niger* commences at the later embryonic period, and the first instar nymphs of *M. niger* have a remarkable pigmented body. As our findings are based on a limited number of hatchlings from eggs laid by one female, more comprehensive observations from different individuals and populations are required, but at the moment such a conspicuous pigmentation would be a singular example reported in plecopteran embryos and hatchlings hitherto known (e.g., Brinck 1949; Khoo 1964; Hynes 1976; Sephton & Hynes 1982; Mtow & Tsutsumi 2021).

It is notable that such an earlier body pigmentation process of *M. niger* occurs. Considering that pigmentation is associated with any given species behaviour, immunity, life history, physiology, and developmental traits (Wittkopp & Beldade 2009), the body pigmentation found in *M. niger* during embryogenesis might be a key to understanding the biology of nymphal period of this species: such kind of body pigmentation pattern might possibly be regarded as the cryptic colouration with respect to escape from predators (Endler 1976). However, our knowledge of bionomics of *M. niger* is only limited to the physical and chemical conditions of streams where the nymphs of this species have been collected (Yokoyama et al. 1990; Inada et al. 1998; Yoshinari 2001; Chino 2011). Additionally, information on nymphal biology, such as ecology and life history of *M. niger*, is almost entirely lacking due to its extremely low population in the field (e.g., Chino 2011; Hanada 2016). Furthermore, *M. niger* has been listed as an endangered species in Nagano and Okayama Prefectures (Nagano Prefecture 2015; Okayama Prefecture 2020) and near-threatened species in Akita Prefecture (Akita Prefecture 2020). To improve our understanding of body pigmentation and the nymphal biology of *M. niger* further, more detailed studies, including embryonic and postembryonic studies and field observations, must be conducted.

Another *Megaperlodes* species, *M. tiunovi*, has matured gray-brown nymphs with a pale pattern (Teslenko 2015), which seems to be similar to other perlodid matured nymphs (e.g., Stewart & Stark 1984; Shimizu et al. 2005; Teslenko & Zhiltzova 2006). However, older nymphs of *M. niger* are different from *M. tiunovi*, i.e., black with the periphery of pronotum yellowish and lateral margin of abdominal segments slightly reddish, which is conspicuous among Japanese stonefly nymphs (Inada et al. 1998; Shimizu et al. 2005). Therefore, further studies are required to determine whether the pigmentation of *M. tiunovi* occurs at the embryonic period.

Acknowledgements – We deeply thank Dr. Toru Kishimoto and Mr. Yasuo Chino for their kind valuable information on *Megaperlodes niger*. We are also grateful to Ms. Mitsuki Mtow for her kind assistance for collecting *M. niger* and ENAGO (www.enago.jp) for the English language review. The present study was supported by a JSPS (Japan Society for the Promotion of Science) KAKENHI: Grant-in-Aid for JSPS Research Fellow, Grant number JP20J00039 to SM.

References

Akita Prefecture. 2020. Threatened Wildlife of Akita Prefecture Red Data Book of Akita Prefecture 2020 Animals II. Akita Kappan Printing, Akita, 161 pp. Available on-line at: https://www.pref.akita.lg.jp/uploads/public/archive_0000048392_00/%E7%A7%8B%E7%94%B0%E7%9C%8C%E7%89%88%E3%83%AC%E3%83%83%E3%83%89%E3%83%87%E3%83%BC%E3%82%BF%E3%83%96%E3%83%83%E3%82%AF2020%E5%8B%95%E7%89%A9%E2%85%A1.pdf [In Japanese].

Berthélemy C. 1979. Mating, incubation period of the eggs, and first larval stages of *Brachyptera braueri* and *Perlodes microcephalus* (Plecoptera). Annales de Limnologie, 15(3): 317–335. Doi: 10.1051/limn/1979015.

Brinck P. 1949. Studies on Swedish Stoneflies (Plecoptera). Opuscula Entomologica Supplementum, 11: 1–250.

Chino Y. 2011. On the emergence period, oviposition date, number of oviposited eggs, egg duration, hatching rate, and duration of adult survival of *Megaperlodes niger*. Bulletin of Chino City Yatsugatake Museum, 19: 8–12. Available on-line at: https://www.city.chino.lg.jp/uploaded/attachment/14226.pdf [In Japanese].

DeWalt R.E., Ower G.D. 2019. Ecosystem services, global diversity, and rate of stonefly species descriptions (Insecta: Plecoptera). Insects, 10(4): 99. Doi: 10.3390/insects10040099

Endler J.A. 1978. A predator's view of animal color patterns, pp. 319–364. In: Hecht M.K., Steere W.C., Wallace B. (eds), Evolutionary Biology, Vol. 11. Plenum Press, New York, London. Doi: 10.1007/978-1-4615-6956-5_5

Fochetti R., Tierno de Figueroa J.M. 2008. Global diversity of stoneflies (Plecoptera; Insecta) in freshwater. Hydrobiologia, 595: 365–377. Doi: 10.1007/s10750-007-9031-3

Hanada S. 2016. Plecoptera, pp. 180–292. In: Maruyama H., Hanada S. (eds), A Field Guide to Japanese Aquatic Insects: Adults of Mayflies, Stoneflies and Caddisflies. Zenkoku Noson Kyoiku Kyokai, Taito-ku, Tokyo. [In Japanese].

Harper P.P. 1979. Observations on the early instars of stoneflies (Plecoptera). Gewässer und Abwässer, 64: 18–28.

Helson G.A.H. 1935. The hatching and early instars of *Stenoperla prasina* Newman. Transactions and Proceedings of the Royal Society of New Zealand, 65: 11–16. Available on-line at: https://paperspast.natlib.govt.nz/periodicals/TPRSNZ1936-65.2.6.3

Hynes H.B.N. 1976. Biology of Plecoptera. Annual Review of Entomology, 21: 135–153. Doi: 10.1146/annurev.en.21.010176.001031

Inada K., Ueno T., Tomita K. 1998. *Megaperlodes niger* in Okayama Prefecture (1). Hyogo Freshwater Biology, 49: 39–45. [In Japanese].

Khoo S.G. 1964. Studies on the biology of stoneflies. Doctral dissertation, Department of Zoology, University of Liverpool, Liverpool, United Kingdom, 161 pp. Available on-line at: https://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.503474

Khoo S.G. 1968a. Experimental studies on diapause in stoneflies. II. Eggs of *Diura bicaudata* (L.). Proceedings of the Royal Entomological Society of London, Series A, General Entomology, 43: 49–56.

Khoo S.G. 1968b. Experimental studies on diapause in stoneflies III. Eggs of *Brachyptera risi* (Morton). Proceedings of the Royal Entomological Society of London, Series A, General Entomology, 43: 141–146.

Kishimoto T. 1997. Comparison of embryonic development among some arctoperlarian species (Plecoptera), pp. 21–25. In: Landolt P., Sartori M. (eds), Ephemeroptera and Plecoptera: Biology-Ecology-Systematics. Mauron + Tinguely & Lachat SA, Fribourg, Switzerland.

Kishimoto T., Ando H. 1985. External features of the developing embryo of the stonefly, *Kamimuria tibialis* (Pictet) (Plecoptera, Perlidae). Journal of Morphology, 183(3): 311–326. Doi: 10.1002/jmor.1051830308

Letsch H., Simon S., Frandsen P.B., Liu S., Machida R., Mayer C., Misof B., Niehuis O., Zhou X., Wipfler B. 2021. Combining molecular datasets with strongly heterogeneous taxon coverage enlightens the peculiar biogeographic history of stoneflies (Insecta: Plecoptera). Systematic Entomology, 46(4): 952–967. Doi: 10.1111/syen.12505

Miller A. 1939. The egg and early development of the stonefly, *Pteronarcys proteus* Newman (Plecoptera). Journal of Morphology, 64(3): 555–609. Doi: 10.1002/jmor.1050640308

Mtow S., Machida R. 2018. Egg structure and embryonic development of arctoperlarian stoneflies: a comparative embryological study (Plecoptera). Arthropod Systematics and Phylogeny, 76(1): 65–86. Available on-line at: https://www.senckenberg.de/wp-content/uploads/2019/07/05_asp_76-1_mtow_65-86.pdf

Mtow S., Machida R. 2021. Thickened serosa and serosal cuticle formed beneath the embryo in eight arctoperlarian stoneflies (Insecta, Plecoptera). Proceedings of the Arthropodan Embryological Society of Japan, 53: 9–13. Available on-line at: http://aesj.co-site.jp/Vol53/2021_Vol.53_9.pdf

Mtow S., Tsutsumi T. 2021. First instar nymphs of two peltoperlid stoneflies (Insecta, Plecoptera, Peltoperlidae). Deutsche Entomologische Zeitschrift, 68(1): 179–188. Doi: 10.3897/dez.68.65540

Murányi D., Hwang J.M. 2015. Just like birdwatching: the first record of *Megaperlodes tiunovi* Teslenko 2015 (Plecoptera: Perlodidae) from Korea. Illiesia, 11(10): 126–129. Available on-line at: http://illiesia.speciesfile.org/papers/Illiesia11-10.pdf

Nagano Prefecture. 2015. Red List of Nagano Prefecture, Animals. Nagano Environmental Conservation Research Institute, Nagano, 234 pp. Available on-line at: https://www.pref.nagano.lg.jp/shizenhogo/kurashi/shizen/hogo/kisyoyasei/redlist/redlist-download.html [In Japanese].

Okayama Prefecture 2020. Insects, pp. 163–306. In: Investigative committee on wild fauna and flora in Okayama Prefecture (ed), Red Data Book of Okayama Prefecture 2020 Animals. Nature

Environment Division, Life and Environmental Department, Okayama Prefecture, Okayama. Available on-line at: https://www.pref.okayama.jp/uploaded/life/656841_5702293_misc.pdf [in Japanese].

Sephton D.H., Hynes H.B.N. 1982. Observations on the first instar nymphs of several Australian stoneflies (Plecoptera). Aquatic Insects, 4(4): 237–252. Doi: 10.1080/01650428209361110

Shimizu T., Inada K., Uchida S. 2005. Plecoptera, pp. 237–290. In: Kawai T., Tanida K. (eds), Aquatic Insects of Japan: Manual with Keys and Illustration. Tokai University Press, Hadanoshi, Japan. [In Japanese].

South E.J., Skinner R.K., DeWalt R.E., Kondratieff B.C., Johnson K.P., Davis M.A., Lee J.J., Durfee R.S. 2021a. Phylogenomics of the North American Plecoptera. Systematic Entomology, 46(1): 287–305. Doi: 10.1111/syen.12462

South E.J., Skinner R.K., DeWalt R.E., Davis M.A., Johnson K.P., Teslenko V.A., Lee J.J., Malison R.L., Hwang J.M., Bae Y.J., Myers L.W. 2021b. A new family of stoneflies (Insecta: Plecoptera), Kathroperlidae, fam. n., with a phylogenomic analysis of the Paraperlinae (Plecoptera: Chloroperlidae). Insect Systematics and Diversity, 5(4): 1–27. Doi: 10.1093/isd/ixab014

Stewart K.W., Stark B.P. 1984. Nymphs of North American Perlodinae genera (Plecoptera: Perlodidae). Great Basin Naturalist, 44 (3): 373–415. Available on-line at: https://scholarsarchive.byu.edu/gbn/vol44/iss3/1

Terry M.D. 2004. Phylogeny of the polyneopterous insects with emphasis on Plecoptera: molecular and morphological evidence. Doctoral dissertation, Department of Integrative Biology, Brigham Young University, Provo, United States, 118 pp. Available on-line at: https://scholarsarchive.byu.edu/etd/1134/

Teslenko V.A. 2015. A new species of *Megaperlodes* Yokoyama et al. 1990 (Plecoptera, Perlodidae) from the South of the Russian Far East. Zootaxa, 3904(4): 553–562. Doi: 10.11646/zootaxa.3904.4.4

Teslenko V.A., Zhiltzova L.A. 2006. Nymphs of the genus *Isoperla* Banks (Plecoptera, Perlodidae) from the Eastern Palaearctic Region. Zootaxa, 1130(1): 1–33. Doi: 10.11646/zootaxa.1130.1.1

Wittkopp P.J., Beldade P. 2009. Development and evolution of insect pigmentation: genetic mechanisms and the potential consequences of pleiotropy. Seminars in Cell and Developmental Biology, 20(1): 65–71. Doi: 10.1016/j.semcdb.2008.10.002

Yokoyama N., Isobe Y., Yamamoto S. 1990. Distribution of *Megaperlodes niger* gen. et sp. nov. (Perlodidae) in Yamagata Prefecture. Abstract of Papers from the 55th Annual Meeting of the Limnological Society of Japan, Yamagata, 65. [In Japanese].

Yoshinari G. 2001. *Megaperlodes niger* in Ibaraki Prefecture. Hyogo Freshwater Biology, 53: 97–99. [In Japanese].

Zwick P. 2000. Phylogenetic system and zoogeography of the Plecoptera. Annual Review of Entomology, 45: 709–746. Doi: 10.1146/annurev.ento.45.1.709