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Laboratory Rearing System for *Ischnura senegalensis* (Insecta: Odonata) Enables Detailed Description of Larval Development and Morphogenesis in Dragonfly

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In an attempt to establish an experimental dragonfly model, we developed a laboratory rearing system for the blue-tailed damselfly, *Ischnura senegalensis*. Adoption of multi-well plastic plates as rearing containers enabled mass-rearing of isolated larvae without cannibalism and convenient microscopic monitoring of individual larvae. Feeding *Artemia* brine shrimps to younger larvae and *Tubifex* worms for older larvae resulted in low mortality, synchronized ecdysis, and normal development of the larvae. We continuously monitored the development of 118 larvae every day, of which 49 individuals (41.5%) reached adulthood. The adult insects were fed with *Drosophila* flies in wet plastic cages, attained reproductive maturity in a week, copulated, laid fertilized eggs, and produced progeny. The final larval instar varied from 9th to 12th, with the 11th instar (56.5%) and the 12th instar (24.2%) constituting the majority. From the 1st instar to the penultimate instar, the duration of each instar was relatively short, mainly ranging from three to 11 days. Afterwards, the duration of each instar was prolonged, reaching 7–25 days for the penultimate instar and 14–28 days for the final instar. Some larvae of final, penultimate and younger instars were subjected to continuous and close morphological examinations, which enabled developmental staging of larvae based on size, shape, and angle of compound eyes and other morphological traits. This laboratory rearing system may facilitate the understanding of physiological, biochemical, and molecular mechanisms underlying metamorphosis, hormonal control, morphogenesis, body color polymorphism, and other biological features of dragonflies.

Key words: dragonfly, damselfly, *Ischnura senegalensis*, laboratory rearing, morphology, life history, ecdysis, metamorphosis

INTRODUCTION

Dragonflies and damselflies (Insecta: Odonata) are relatively large, actively flying, diurnal, and colorful insects in which vision, mediated by highly-developed compound eyes, is the primary sensory modality (Corbet, 1999; Futahashi, 2016). Their elaborate territorial, offensive, mating and reproductive behaviors, in combination with their sexually, temporally and ecologically polymorphic body colors and patterns, have attracted the attention of biologists, resulting in a large number of ecological, behavioral and evolutionary studies on dragonflies (Tillyard, 1917; Corbet, 1999; Córdoba-Aguilar, 2008). Physiological, biochemical, and molecular genetic studies on dragonflies, however, have been disproportionally scarce, mainly due to the lack of model dragonfly species that are experimentally tractable and amenable to rearing in laboratory conditions (Bybee et al., 2016). Because larval dragonflies are avid predators, feeding them is laborious, and mass-rearing them is particularly difficult due to cannibalism. Because adult dragonflies are active fliers and their sexual maturation takes a relatively long time, it is very difficult to keep them in laboratory conditions that enable the reaching sexual maturity (Corbet, 1999, Chapter 8).

Hence, although many biologists have described a variety of life history traits of diverse dragonflies, the majority of such reports are neither thorough nor systematic but rather fragmentary in general (but see Grieve, 1937) owing to the difficulty in continuously rearing the dragonflies. An interesting but poorly established aspect in the dragonfly biology is the number and variation of larval instars. In contrast to 3–8 larval instars observed in the majority of insects, dragonflies (Odonata) and mayflies (Ephemeroptera) are known to exhibit exceptionally large numbers, usually more than 10, of larval instars (Corbet, 1999, Chapter 7.2; Esperk et al., 2007). Notably, as many as 34 and 24 larval instars have

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been recorded in the mayfly *Leptophlebia cupida* and the dragonfly *Trithemis furva*, respectively (Clifford et al., 1979; Corbet, 1999, Chapter 7.2). In some dragonflies, variation in larval instars has been reported on the basis of head width of field-caught larvae (Norling, 1984; Aoki, 1999), or larval rearing in the laboratory (Balfour-Browne, 1909; Grieve, 1937; Pellerin and Pilon, 1977; Rivard and Pilon, 1977; Pilon and Rivard, 1979; Pilon and Fontaine, 1980; Pilon and Masseau, 1983; Leggott and Pritchard, 1985; Mathavan, 1990; Corbet, 1999, Chapter 7.2). In several dragonflies, morphological traits, including size of body parts, such as head width and body length, and number of body parts, such as segments and bristles, have been measured and compared among larval instars (Miyakawa, 1969; Pellerin and Pilon, 1977; Rivard and Pilon, 1977; Pilon and Rivard, 1979; Pilon and Fontaine, 1980; Pilon and Masseau, 1983; Leggott and Pritchard, 1985; Corbet, 1999, Chapter 7.2; Di Giovanni et al., 2000; Goretti et al., 2001; Velásquez et al., 2008; Casallas-Mancipe et al., 2012). Some morphological changes prior to eclosion have been reported, mostly focusing on the shapes of compound eyes and wing sheaths (Grieve, 1937; Corbet, 1957; Eller, 1963; Miyakawa, 1969; Norling, 1976; Andries, 1979; Norling, 1984; Corbet and Prosser, 1986). However, it is not easy to integrate these previous reports into a coherent picture, since these data are usually fragmentary, collected from different species under different conditions, and not necessarily recorded in a standardized/systematic way. Many life history traits, such as the precise timing of these morphological changes, have not been described in sufficient detail (Eller, 1963; Norling, 1984). Due to the difficulty in mass rearing and their relatively long lifespan, basic biological aspects, such as number of larval instars, duration of each larval instar, and larval morphological changes, have not been established in most dragonfly species.

Thanks to recent technological innovations, high-quality genomic and transcriptomic data can now easily be obtained from non-model organisms, including dragonflies (Chauhan et al., 2014; Futahashi et al., 2015; Chauhan et al., 2016; Lancaster et al., 2016; Suvorov et al., 2016; Ioannidis et al., 2017), and powerful molecular genetic tools, such as RNA interference and CRISPR/Cas9, are becoming applicable to non-model organisms, including dragonflies (Chen et al., 2016; Okude et al., 2017). Thus, there is a need for a model dragonfly species, which ideally should be maintainable in the laboratory and amenable to physiological, biochemical, and molecular genetic analyses. In this context, some damselfly species of the suborder Zygoptera, which are small-sized, of short generation time, capable of continuous reproduction without hibernation, less active (so as not to damage themselves in cages), and willing to accept laboratory diets, seem appropriate for that purpose.

Here, we established a laboratory rearing system for the blue-tailed damselfly *Ischnura senegalensis* (Rambur, 1842) (Odonata: Zygoptera: Coenagrionidae) (Fig. 1). We selected *I. senegalensis* as a model species for the following reasons: (i) it is among the most common dragonflies in Japan (Ozono et al., 2012); (ii) it is naturally multivoltine, with a couple of generations per year (Ozono et al., 2012); (iii) its generation time, about three months, is among the shortest ever reported in a dragonfly (Yoshida, 2014); (iv) there are previous reports on rearing and breeding of several *Ischnura* species (Van Gossum et al., 2003; Abbott and Svensson, 2005; Takahashi and Watanabe, 2010; Piersanti et al., 2015); (v) there are previous studies on behavioral ecology and female color polymorphism of *Ischnura* species (Corbet, 1999, Chapter 8; Córdoba-Aguilar, 2008; Svensson et al., 2009; Takahashi et al., 2014; Futahashi, 2016; Bybee et al., 2016); and (vi) transcriptomic studies have been conducted on several *Ischnura* species (Chauhan et al., 2014; Futahashi et al., 2015; Chauhan et al., 2016; Lancaster et al., 2016; Suvorov et al., 2016). In the present study, by making use of the laboratory rearing system, we continuously monitored the development of as many as 118 larvae of *I. senegalensis* every day, of which 49 individuals (41.5%) attained adulthood. These data provided unprecedentedly detailed descriptions on ecdysis, eclosion and death events, and in particular, morphological changes during the final (F-0), penultimate (F-1) and juvenile (5^{th} – 7^{th}) larval instars of this dragonfly species.

MATERIALS AND METHODS

Terminology

In order to describe a developmental stage between molts, we adopted the term “instar” that is commonly used among diverse insects, although the term “stadium” has often been used in dragonflies (Corbet, 1999, Chapter 7.2). In this paper, we adopted the term “larva”, not “nymph”, following the convention used in the majority of previous studies on dragonflies (e.g., Tillyard, 1917; Corbet, 1999, Chapter 7.2). In hemimetabolous insects, a “pronymph” stage generally exists just after hatching (Truman and Riddiford, 1999), which has been designated as the “prolarva” stage in dragonflies. In this study, we could observe only a few prolarvae because of the short duration of the prolarval period for roughly two minutes (Shimura, 2006). Following the majority of the published literature on dragonflies, we describe the final larval instar as F-0 or F (= Final), and the penultimate larval instar as F-1 (= F minus 1). We introduced these terms because the final larval instar is generally not fixed in dragonflies. For each instar, the “day 1” denotes the day on which ecdysis occurred.

Insect sampling

Adult females of *I. senegalensis* were collected in Tsukuba, Ibaraki, Japan on 20 May, 26 May, and 23 June 2016. The adult females exhibited two color morphs called the gynochrome and the androchrome (Fig. 1E), where the former is genetically dominant over the latter (Takahashi and Watanabe, 2010; Ozono et al., 2012). On account of a previous study on a closely related species *I. elegans*, which reported that the larval developmental period is different between the female morphs (Abbott and Svensson, 2005), we used only gynochrome females in this study.

Insect rearing

In order to collect eggs, reproductively-mature females were individually introduced into each petri dish with fully-wet filter paper, where some, if not all, of them laid eggs into the filter paper within two days at room temperature (Fig. 2A). The duration of egg period was 9–10 days (Fig. 3A), as previously reported (Yoshida, 2014), in which pigmented eyespots became visible a few days before hatching (Fig. 1B). After hatching, newborn larvae were transferred to each well of 48-well plastic plates by a dropper for individual rearing (Fig. 2B). As the larvae grew, they were transferred to 24-well plastic plates, and then to 12-well plastic plates (Fig. 2C). The rearing plates were placed in an incubator at 25°C under a long day condition of 16 h light and 8 h dark. We examined five groups of newborn larvae, namely the groups a, b, c, d and e (Fig. 3A; Supplementary

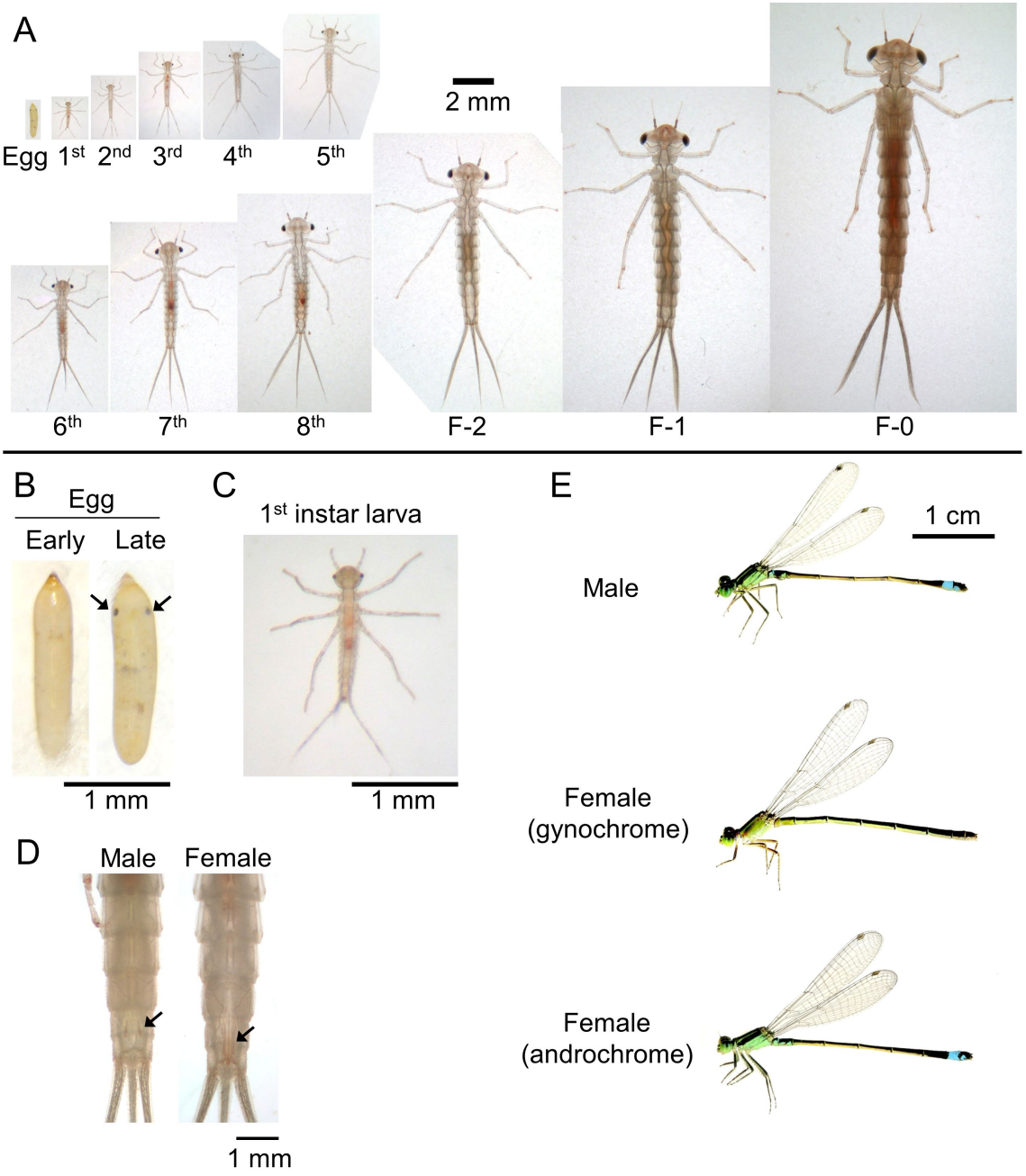


Fig. 1. Developmental stages and morphology of *I. senegalensis*. **(A)** From egg to final (F-0) larval instar. **(B)** Early and late eggs, in which arrows indicate eyespots. **(C)** A 1st instar larva. **(D)** Sex-specific traits on the ventral side of the larval abdominal tip (arrows). **(E)** Adult male, gynochrome female, and androchrome female.

Figure S1), each of which consisted of a clutch of larvae derived from the same gynochrome female. Note that these groups were derived from three females: groups a and c from female 1, group b from female 2, and groups d and e from female 3. To minimize the effects of nutritional fluctuation, we carefully supplied almost the same amount of food to the larvae of the same experimental group at the same age every day using a micropipette under a stereoscopic microscope. The small young larvae were fed with *Artemia* brine shrimps until 1 August 2016 for groups a, b and c, and until 10 August 2016 for groups d and e (Fig. 3A). Subsequently, the larger, older larvae were fed with *Tubifex* worms. We inspected all the rearing wells under a stereoscopic microscope every day, collected shed skins and recorded molting dates. When the wing sheaths of F-0 instar larvae were recognizably expanded, the larvae were individually transferred to a plastic cage (10 cm × 15 cm, 3 cm water depth) covered with a disposable non-woven mesh for assisting eclosion (Fig. 2D, E). Initially we also used 50 ml plastic tubes containing a wood stick and water as an arena for eclosion (Fig. 2F), but the system resulted in frequent failure of normal eclosion due to limited space and instability for the larvae. All the newly-emerged adult females were gynochrome. After eclosion, the teneral adults were kept in the cages for a few days, and then transferred to a larger plastic cage (17 cm × 27 cm, 18 cm high; purchased at a 100-yen store) (Fig. 2G, H). The bottom of the cage was covered with wet sponges, the wall of the cage was covered with a disposable non-woven mesh, two *Drosophila*-culturing plastic tubes were

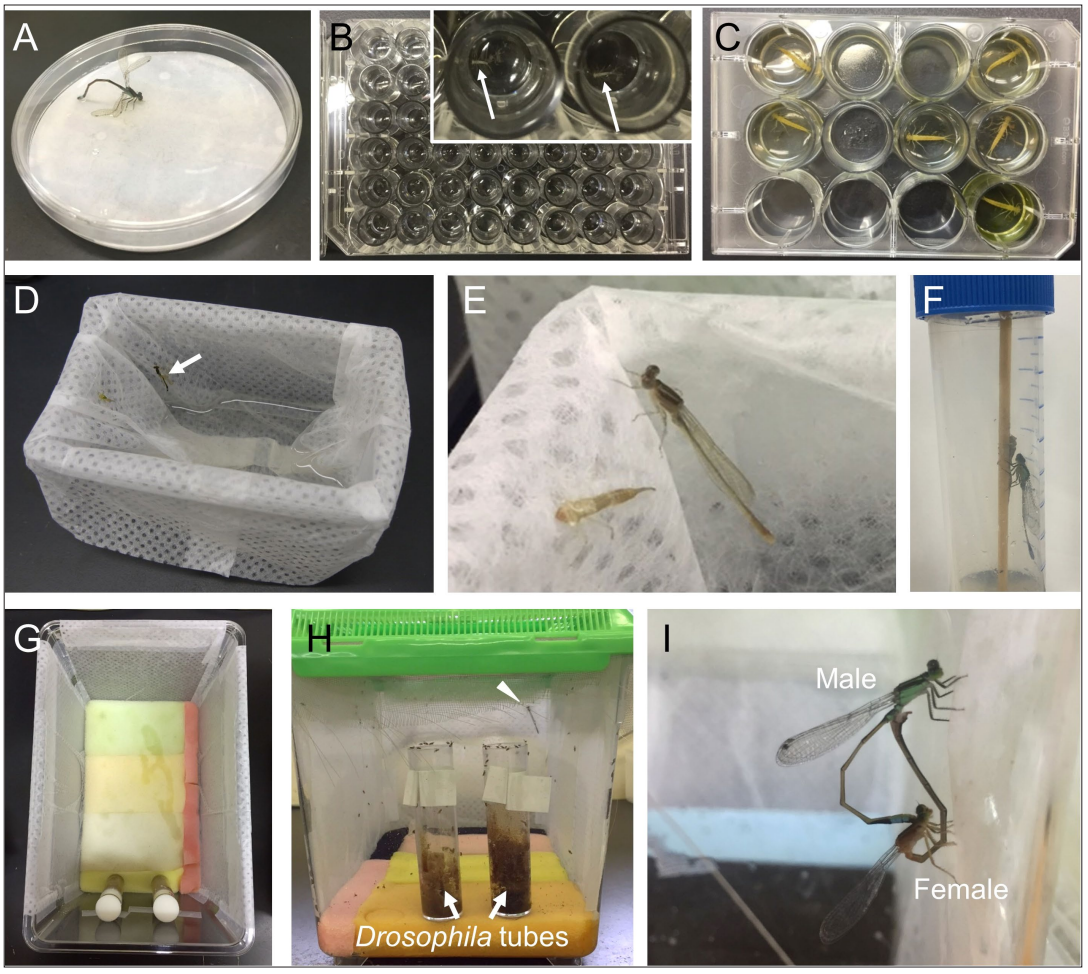


Fig. 2. Laboratory rearing system for *I. senegalensis*. **(A)** An adult female laying eggs on filter paper in a petri dish. **(B)** Individual rearing of young larvae in a 48-well plastic plate. Arrows in the inset indicate larvae. **(C)** Individual rearing of F-0 instar larvae in a 12-well plastic plate. **(D)** A cage for adult eclosion. With the lid removed, an eclosing adult is seen (arrow). **(E)** A magnified view of a newly-eclosed adult and a shed skin in the eclosion cage. **(F)** An example of adult eclosion in a 50 ml plastic tube. **(G)** Top view of a cage for adult rearing whose lid is removed. **(H)** Side view of a cage for adult rearing. Arrows and an arrowhead indicate *Drosophila*-rearing tubes and an adult insect, respectively. **(I)** A mating pair of *I. senegalensis* in the cage.

placed in the cage as food sources for the adult insects, and the lid of the cage was covered with a net to prevent the insects from escaping (Fig. 2G, H). About a week after the adult emergence, mating adult pairs were observed in the cage (Fig. 2I), and some females subsequently laid eggs when transferred to petri dishes as described above (Fig. 2A).

Morphometry

In order to investigate morphological changes during larval development in detail, six F-0 instar larvae, three F-1 instar larvae, and three juvenile (from 5th to 7th) instar larvae were photographed every day using a stereoscopic microscope SZ-6850T (Relyon) with a digital high definition microscope camera TrueChrome Metrics (Relyon), or a stereoscopic microscope S8APO (Leica Microsystems) with a digital high definition microscope camera MC120HD (Leica). Larval sexing was performed on the basis of sex-specific morphological traits seen on the ventral side of the abdominal tip (Fig. 1D). These sex-specific traits were clearly recognized at least after F-3 instar larvae, but could not detected in 5th, 6th, and 7th instar larvae. The head width and body length (not including antennae and caudal gills) were measured on the digital images. To determine the area of the larval compound eye, a circular region enclosing the eye was selected manually and the threshold was set on the basis of Otsu's discriminant analysis (Otsu, 1979), followed by the opening and closing image operations to eliminate noise pixels. For measurement of the angle of compound eyes (see Fig. 8A), principal component analysis was applied to the area, which estimated the main axis of the compound eye as the first component. The average angle of the compound eyes was calculated for the left and right compound eyes. All the image processing operations and the measurements were conducted on the software Natsumushi version 1.00 (https://sites.google.com/site/mtahashilucanid/program/natsumushi) developed by M.T. using Visual C++ 2005 (Microsoft). Statistical analysis was performed by R software (version 3.3.1) (R Core Team, 2016).

RESULTS AND DISCUSSION

Number, duration and variation of larval instars

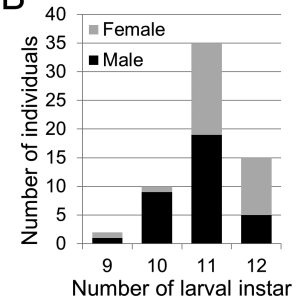
We established a laboratory rearing system for *I. senegalensis* (Fig. 2), which enabled us to investigate the development and life history of the dragonfly species in detail. We recorded ecdysis, eclosion and death events of 118 individuals, which represented five experimental groups corresponding to five clutches derived from field-collected gynochrome females, every day for up to 116 days (Fig. 3A;

Supplementary Figure S1). Of 118 newborn larvae we monitored, 62 individuals (34 males and 28 females; 52.5%) reached the final larval (F-0) instar, of which 49 individuals (28 males and 21 females; 41.5%) attained adulthood. Notably, the F-0 instar was not constant but varied: 2 larvae (3.2%) at the 9th instar, 10 larvae (16.1%) at the 10th instar, 35 larvae (56.5%) at the 11th instar, and 15 larvae (24.2%) at the 12th instar (Fig. 3B). The variation was observed even within the same clutch (Fig. 3C) as previously reported in other dragonflies (e.g. Corbet, 1999, Chapter 7.2). The number of larval instar was slightly larger in females (11.3 ± 0.7, n = 28) than in males (10.8 ± 0.7, n = 34) (Mann-Whitney U test, P = 0.013). From the 1st instar to the F-2 instar, the duration of each instar was relatively short, mainly ranging from three to 11 days (Fig. 4), showing no significant differences between males and females at any larval instar (Mann-Whitney U

A

	Collected	Oviposition	Hatching	Change food
a	26 May 2016	28 May 2016	6 Jun. 2016	1 Aug. 2016
b	20 May 2016	21 May 2016	30 May 2016	1 Aug. 2016
c	26 May 2016	28 May 2016	6 Jun. 2016	1 Aug. 2016
d	23 Jun. 2016	25 Jun. 2016	5 Jul. 2016	10 Aug. 2016
e	23 Jun. 2016	25 Jun. 2016	5 Jul. 2016	10 Aug. 2016

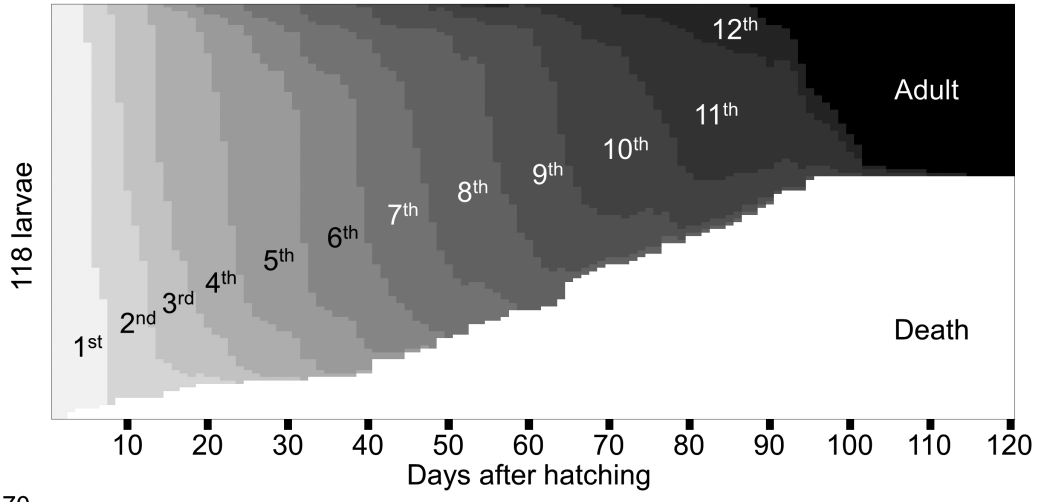
B



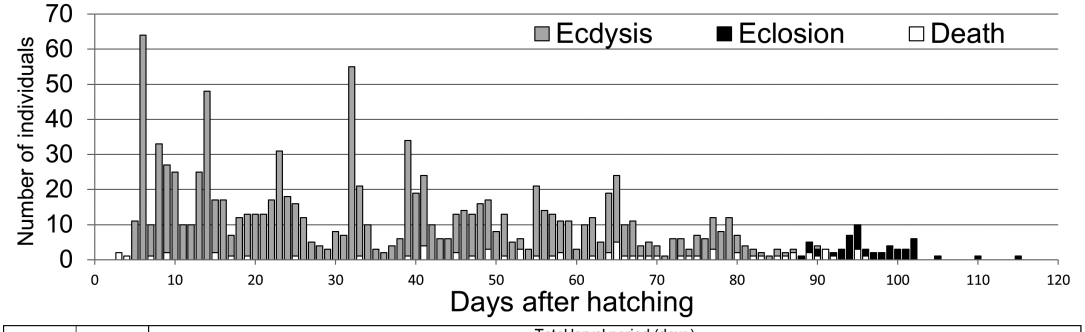
C

		Numbers of larval instar			
		9	10	11	12
a	Male	1	4	6	1
	Female	1		10	8
b	Male			1	1
	Female				1
c	Male		2	4	
	Female			1	
d	Male		2	5	3
	Female		1	2	
e	Male		1	3	
	Female			3	1

D



E



F

Instar No.	Sex	Total larval period (days)																												
		88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115
9th	Male			1																									
	Female									1																			
10th	Male		1				1	2	2			1				1													
	Female												1																
11th	Male		1				1	3	5				1	2	1	1			1										
	Female		1	1		1	1	2		1	1	1	1	1	1	1								1					
12th	Male	1											1			1													
	Female										1				1	2													1

Fig. 3. Life history traits monitored during the larval development of *I. senegalensis*. **(A)** Information on five experimental groups a, b, c, d and e. Each group consists of a clutch of larvae derived from a single adult female. **(B)** Variation of the final instar ranging from 9th to 12th. **(C)** Variation of the final instar within and between the experimental groups a, b, c, d and e. **(D)** Ecdysis and eclosion patterns during the development of 118 larvae. **(E)** Daily record of ecdysis, eclosion and death events during the development of 118 larvae. **(F)** Total larval periods of F-0 instar larvae that reached adulthood.

test, $P > 0.05$). Afterwards, the duration of each instar was remarkably prolonged, reaching 7–25 days for the F-1 instar and 14–28 days for the F-0 instar (Fig. 4). The total larval period ranged from 88 to 115 days (Fig. 3D–F), which exhibited no significant differences between males and females at any larval instar (Mann-Whitney U test, $P > 0.05$; Steel-Dwass test, $P = 0.15$).

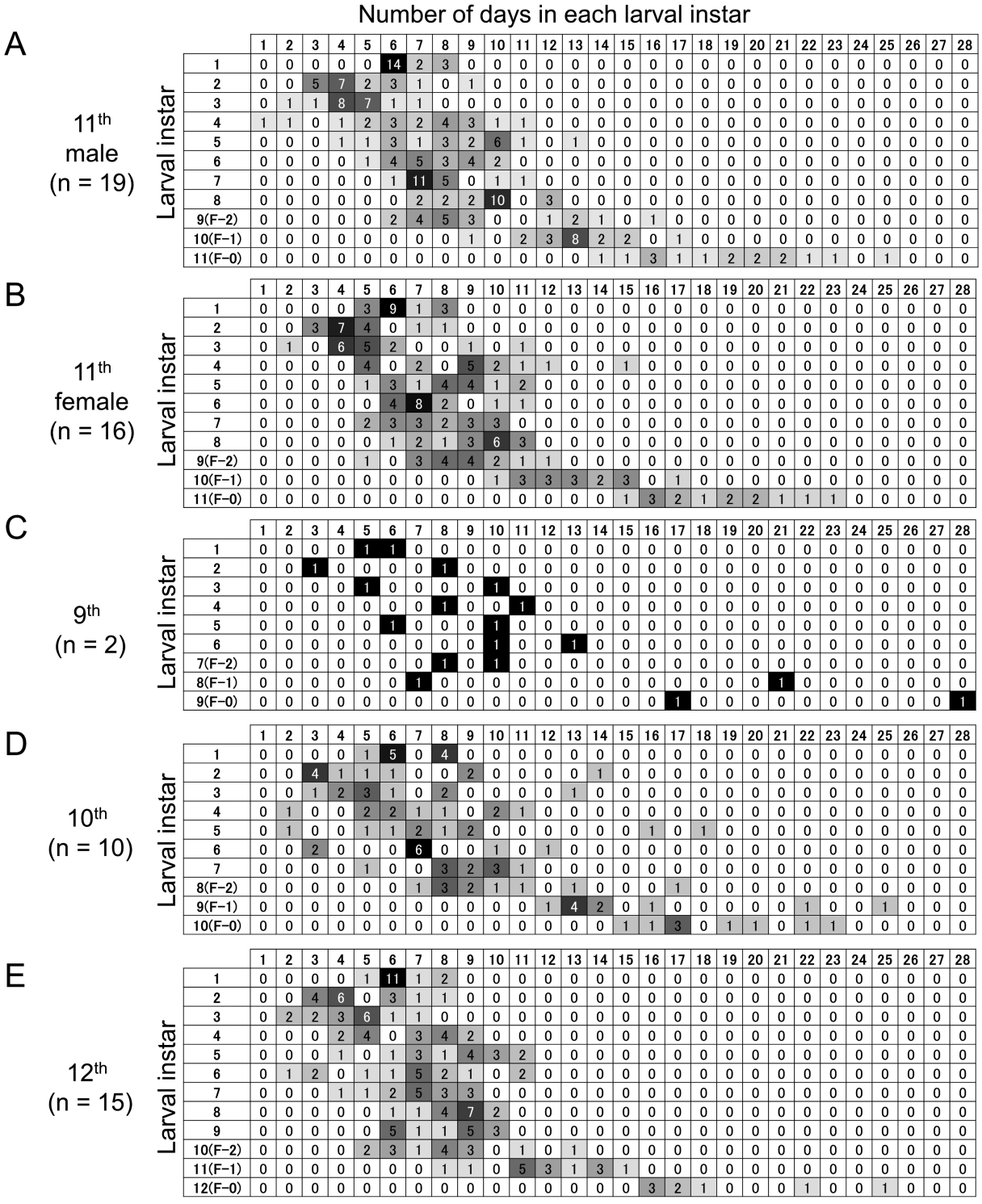


Fig. 4. Duration of each instar during the larval development of *I. senegalensis*. **(A)** Male larvae whose final instar is 11th ($n = 19$). **(B)** Female larvae whose final instar is 11th ($n = 16$). **(C)** Larvae whose final instar is 9th ($n = 2$). **(D)** Larvae whose final instar is 10th ($n = 10$). **(E)** Larvae whose final instar is 12th ($n = 15$). Note that male larvae and female larvae are pooled in **(C–E)**. The colors of the boxes correspond to the proportion of the number of individuals among the same larval instar larvae (black means high proportion).

Morphological changes in F-0 instar larvae

In particular, we focused on morphological changes in the F-0 instar prior to eclosion. Six F-0 instar larvae (three males and three females) were photographed every day, which were subjected to detailed morphological observations and measurements (Fig. 5; Supplementary Figure S2A). Considering that the average duration of the F-0 instar was approximately 19 days (Fig. 4), we first examined the

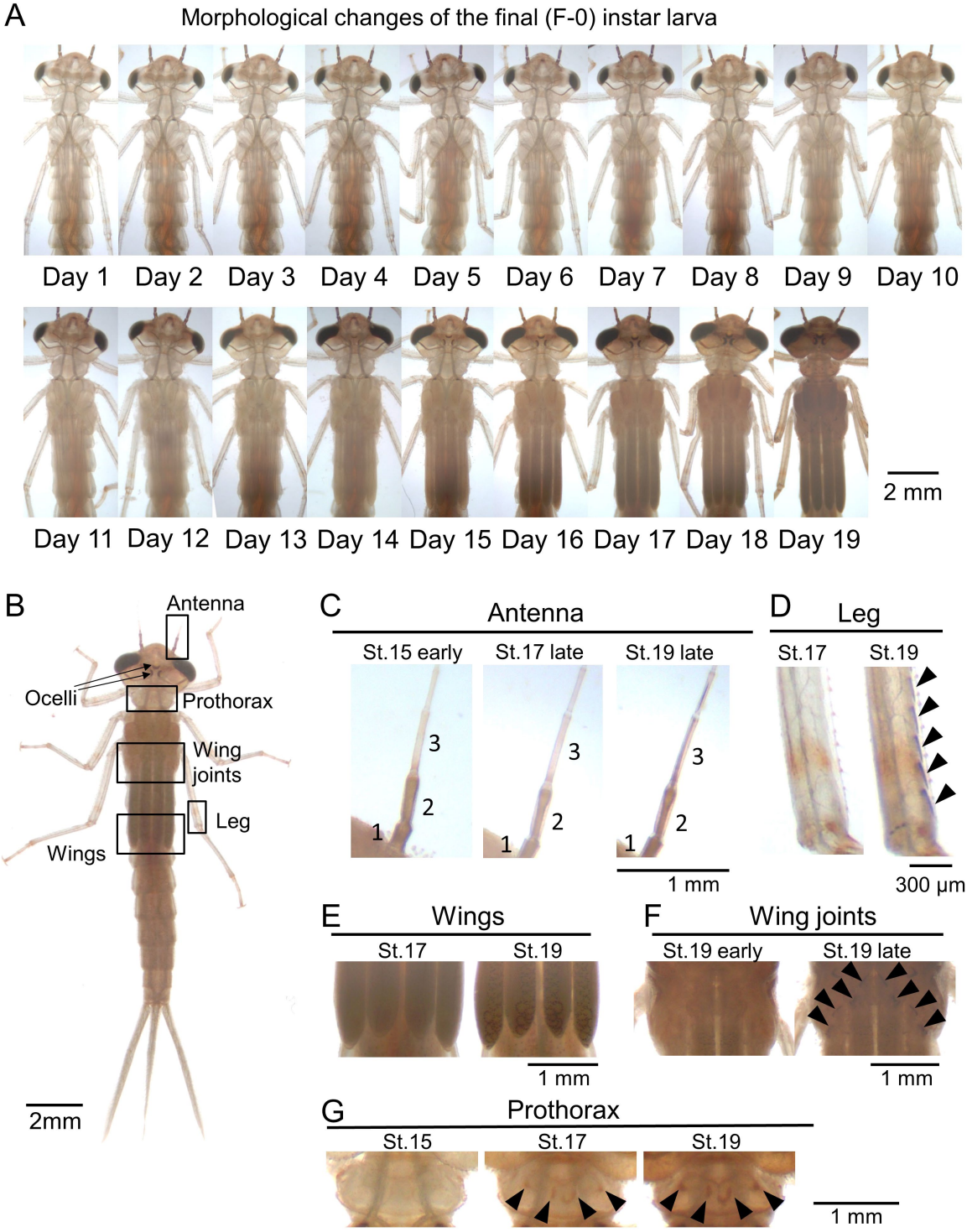


Fig. 5. Morphological changes in the final (F-0) instar larvae of *I. senegalensis*. **(A)** Daily photos of the head-thorax region of a female larva. **(B)** A photo of the whole body, on which body parts are highlighted by rectangles and arrows. **(C–G)** Magnified images of the body parts: **(C)** an antenna, on which antennal segments are indicated by numbers; **(D)** a leg (= hind femur); **(E)** wings; **(F)** wing joints; and **(G)** prothorax. In (D), (F) and (G), pigmented regions are highlighted by arrowheads. Note that the developmental staging (from stage 1 to stage 19) was defined on the basis of the age (from day 1 to day 19) of the individual shown in (A). Also see Fig. 6.

timing of morphological changes in a female larva (no. 2) whose duration of the F-0 instar was 19 days (Fig. 5A). The most evident changes were observed in the shape and size of the compound eyes. The compound eyes gradually enlarged from day 1 to day 11, and expanded rapidly toward the dorsal side from day 12 (designated as stage 12) (Fig. 5A). The dorsal expansion of the compound eyes completed by day 15 (stage 15) (Fig. 5A). From day 17, the compound eyes started to expand posteriorly (stage 17), and the expansion completed by day 19 (stage 19) (Fig. 5A). In parallel with the changes in the compound eyes, a number of morphological changes were observed: wing sheaths exhibited remarkable development from stage 12 to stage 17 (Fig. 5A); the antennal segment 2 became narrower from stage 15 to

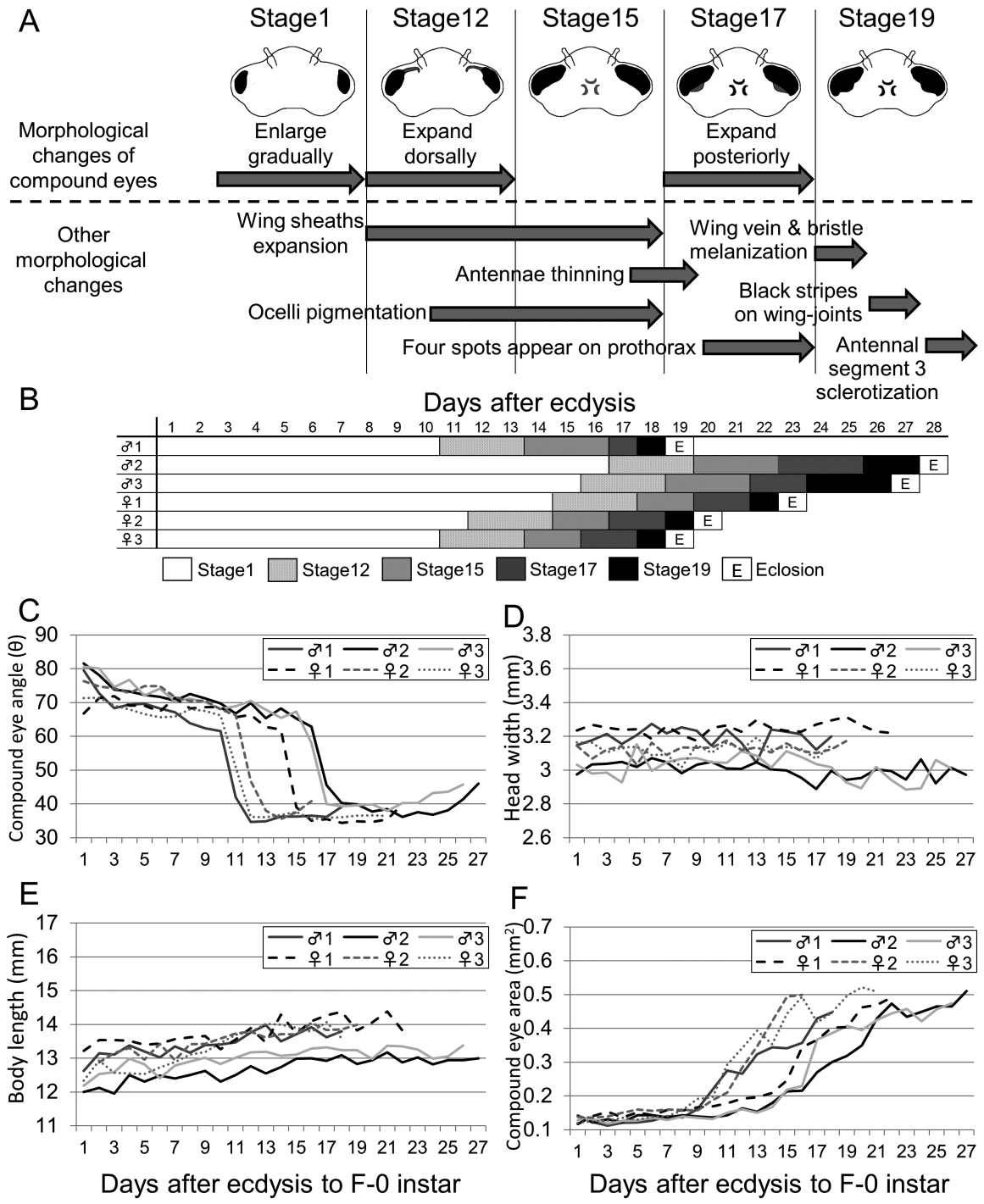


Fig. 6. Schematic illustration of morphological changes in the final (F-0) larval instar of *I. senegalensis*. **(A)** Summary of developmental stages, morphological changes in compound eyes, and morphological changes in other body parts. **(B)** Developmental stages of six larvae at the F-0 instar we examined. **(C–F)** Daily changes in compound eyes and other morphological traits of six larvae at the F-0 instar: **(C)** compound eye angle; **(D)** head width; **(E)** body length; and **(F)** compound eye area.

stage 17 (Fig. 5C); the antennal segment 3 was sclerotized at stage 19 (Fig. 5C); pigmentation around ocelli started at stage 12 and became prominent at stage 15 (Fig. 5A); at stage 17 onward, pigmented patterns occurred on bristles of the legs, on wing sheaths as circles, on wing joints as four pairs of slant lines, and on prothorax as two pairs of curved lines (Fig. 5A and D–G); and others. Figure 6A shows a schematic illustration of the morphological changes in the F-0 instar, which enables morphological staging of the larval development toward adult emergence in *I. senegalensis*. Note that these developmental changes occurred in the same order in all the six individuals we examined (Fig. 6B; Supplementary Figure S2A). It should also be noted that larval feeding behavior stopped from stage 17 onward.

Morphological changes in F-1 instar larvae

Next, we examined morphological changes in the F-1 instar based on daily photos of three larvae (a male and two females) (Fig. 7A; Supplementary Figure S2B). We observed that wing sheaths were flat and inconspicuous in the early F-1 instar, whereas they expanded and became prominent a few days before ecdysis to the F-0 instar (Fig. 7A). This pattern was consistently observed in all three F-1 instar larvae we inspected (Fig. 7B; Supplementary Figure S2B). We also found that the angle of the compound eyes changed during the F-1 instar: gradually tilting to the body midline until ecdysis to the F-0 instar (Figs. 7A, 8A, B). During the developmental course of the F-1 instar larvae, head width was nearly constant whereas body length and size of compound eyes steadily increased (Figs. 7A, 8C–E). The progressive tilt of the compound eyes may be caused by deformation of the growing larval organs packed and constrained in the rigid cuticular head capsule, an idea that is supported by the observation that the tilted angle of the compound eyes recovered upon ecdysis to the F-0 instar, coincident with discontinuous enlargement of the head width (Fig. 8B, C). Note that similar morphometric patterns were also observed in the developmental course of the F-0 instar larvae: progressive tilt of the compound eyes (Fig. 6C), almost constant head width (Fig. 6D), and increasing body length and compound eye size (Fig. 6E, F).

Morphological changes in younger instar larvae

Finally, we examined morphological changes in the younger instars based on daily photos of three larvae (two males and a female) from the 5th instar to the 7th instar (Supplementary Figure S2C). The patterns of morphological changes in the younger instars were largely similar to those in the F-1 instar: angular tilt of the compound eyes gradually proceeded during the intermolt period and recovered upon ecdysis (Fig. 8F); head width was almost constant during the intermolt period and discontinuously increased upon ecdysis (Fig. 8G); body length and size of compound eyes gradually and steadily increased during the developmental course (Fig. 8H, I).

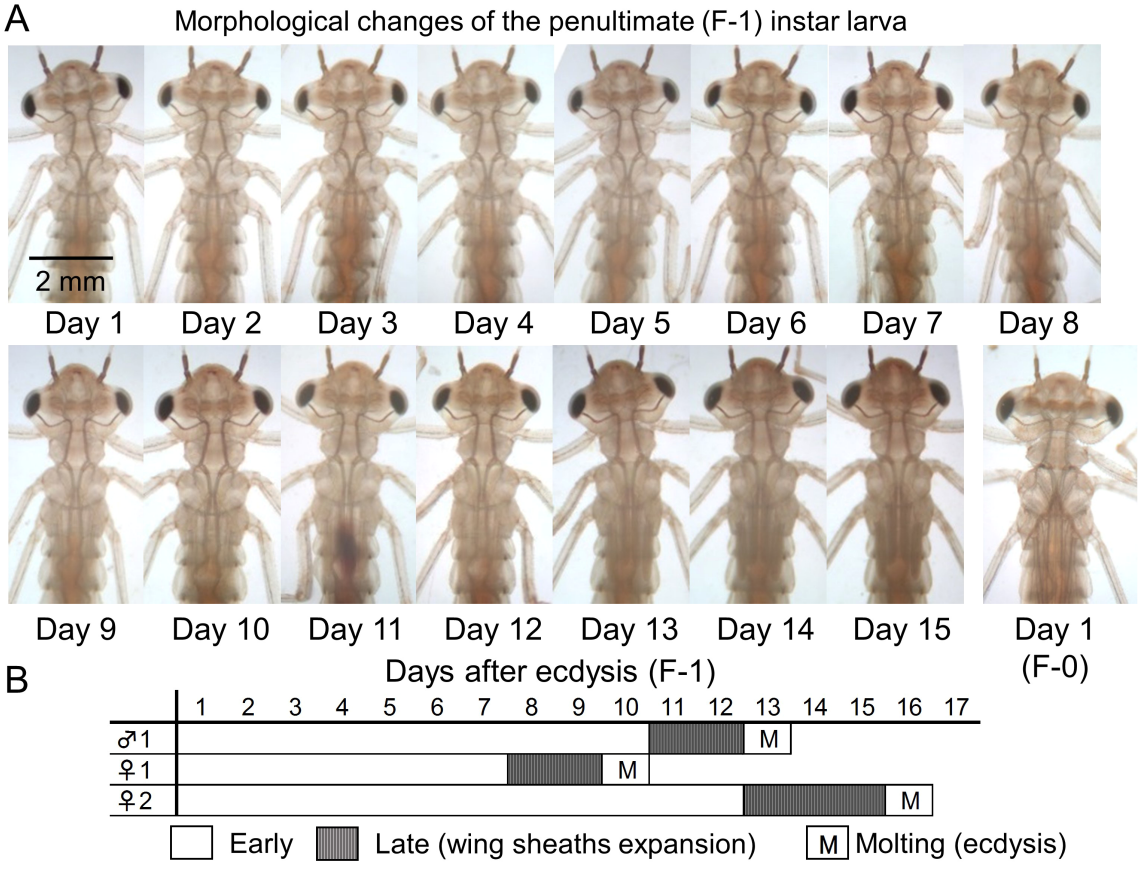


Fig. 7. Morphological changes in the penultimate (F-1) instar larvae of *I. senegalensis*. **(A)** Daily photos of the head-thorax region of a female larva. **(B)** Developmental stages observed in the three F-1 instar larvae we examined.

Production of progeny

Of the 49 adult insects emerged in the laboratory, most individuals were killed by accident or cannibalism (= occasional consumption of males by females), and only 10 adult insects attained sexual maturity. Around 12 days after eclosion, we observed two mating pairs in the group a, and one mating pair in the group d. On the next day, these mated females laid eggs on wet filter paper in petri dishes, and 10 to 12 days later, many hatchlings (> 200 in total) were found in the petri dishes, which indicated that the eggs of the labo-

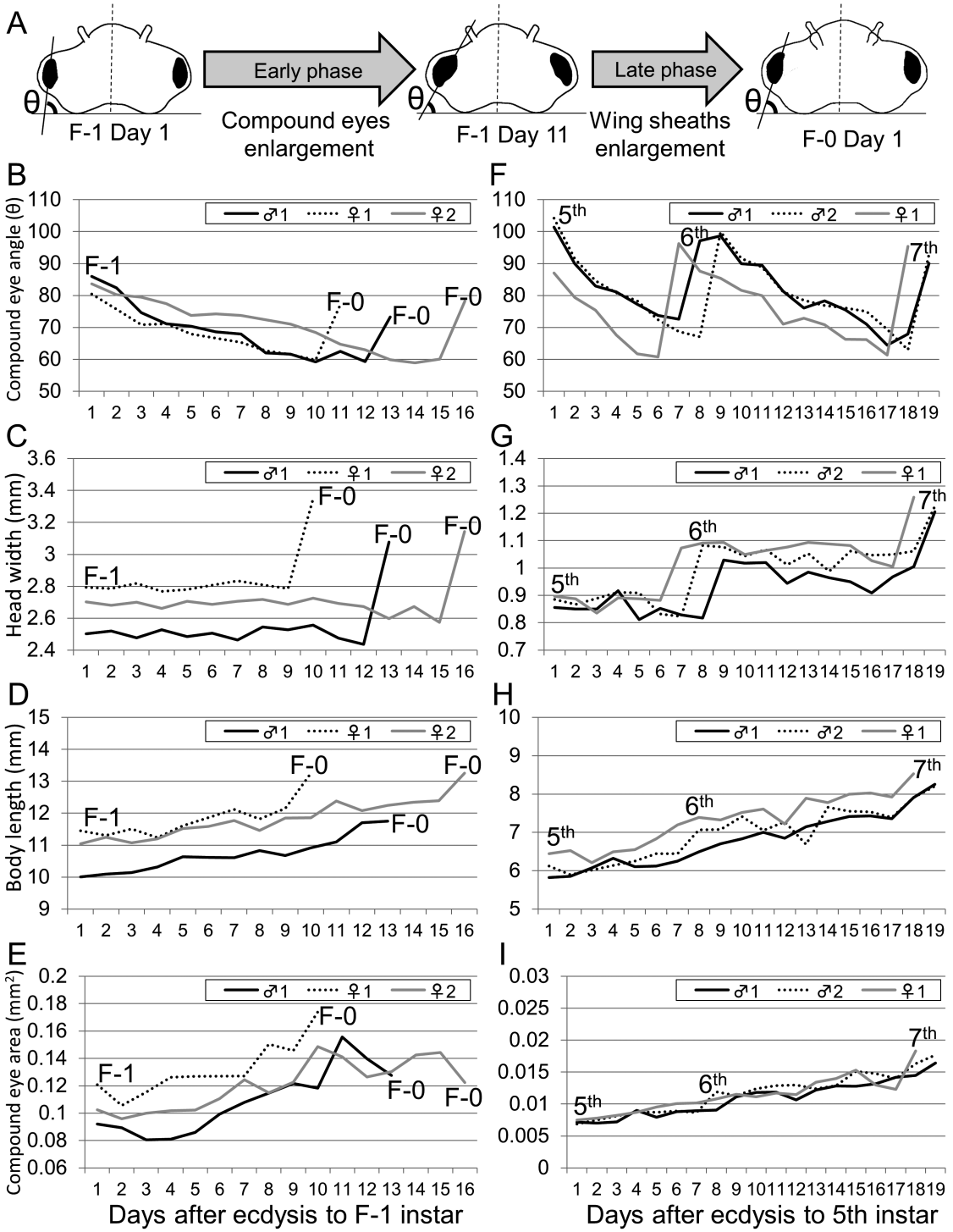


Fig. 8. Angle and size of compound eyes, head width and body length during the larval development of *I. senegalensis*. **(A)** Definition of the compound eye angle, and schematic illustration of changes in shape and size of compound eyes during the F-1 instar. **(B–E)** Daily changes in compound eyes and other morphological traits of three larvae at the F-1 instar: **(B)** compound eye angle; **(C)** head width; **(D)** body length; and **(E)** compound eye area. **(F–I)** Daily changes in compound eyes and other morphological traits of three larvae at the 5th to 6th instar: **(F)** compound eye angle; **(G)** head width; **(H)** body length; and **(I)** compound eye area.

ratory-reared females were normally fertilized and viable. These newborn larvae in the second generation were not handled individually but reared in several plastic containers, to which foods were provided every two or three days. Despite the rough rearing practices, 11 adult insects (eight males and three females) emerged finally.

Conclusion and perspective

In conclusion, we established a useful laboratory rearing system for a dragonfly species, *I. senegalensis*. Adoption of multi-well plastic plates as rearing containers enabled mass-rearing of isolated larvae without the problem of cannibalism and allowed convenient microscopic monitoring of individual larvae. The laboratory foods, *Artemia* brine shrimps and *Tubifex* worms, are easily available commercially and inexpensive, and supported the larval growth very well. Our rearing attained a high adult emergence rate over 40%, the adult insects mated and laid eggs in a plastic cage, and the eggs hatched and developed to adult insects of the next generation. To our knowledge, this study provides the most detailed and comprehensive descriptions of larval development and morphogenesis in a dragonfly, which we hope will provide a firm basis underpinning a variety of biological studies on dragonflies in the future. Dragonflies constitute one of the most basal lineages of the hemimetabolous insects, and are often regarded as the most ancestral group of the winged insects (Misof et al., 2014). Their metamorphosis entails transition from aquatic lifestyle to terrestrial/aerial lifestyle, upon which their morphology, physiology and ecology exhibit drastic changes (Tillyard, 1917; Corbet, 1999, Chapter 7). However, endocrinological and molecular biological aspects of dragonfly's metamorphosis remain very poorly understood: even juvenile hormone is still uncharacterized, and identity of hormone-producing organs remains still under debate (Schaller, 1989). Their vivid body colors and patterns, which often exhibit sexual, temporal and ecological polymorphisms, have been the major subject of ecological, behavioral and evolutionary studies, but endocrinological, physiological, biochemical, and molecular controlling mechanisms over the colors and patterns are still to be explored (reviewed in Bybee et al., 2016; Futahashi, 2016). In the present era when genomic, transcriptomic, and genome editing technologies are becoming applicable to non-model organisms, the experimental dragonfly model developed in this study may provide clues to the long-lasting unsolved questions concerning the biology of dragonflies.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

GO and RF designed the research. GO mainly performed the research. MT assisted morphometric analyses. GO, RF and TF wrote the paper.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available online (URL: http://www.bioone.org/doi/suppl/10.2108/zs170051).

Supplementary Figure S1. Raw data of ecdysis, eclosion and death events observed with 118 larvae of *I. senegalensis*.

Supplementary Figure S2. Photos used for analysis of morphological changes in the larval development of *I. senegalensis* in this study.

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