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ORIGINAL PAPER

Oviposition-like central pattern generators in pregenital segments of male and female grasshoppers

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Abstract

Grasshoppers produce an extraordinary oviposition behavior that is associated with multiple specializations of the skeletal and neuromuscular systems in the posterior abdomen, including a central pattern generator (CPG) in the female's terminal abdominal ganglion. Two pairs of shovel-shaped appendages, the ovipositor valves on the abdomen tip, excavate the soil for deposition of eggs. By contrast, the sexually monomorphic pregenital region of the abdomen is without appendages. Morphological homologues of ovipositor muscles and efferent neurons in the eighth abdominal segment are nevertheless present in pregenital segments of males and females. In both sexes, a robust rhythmic motor program was induced in pregenital segments by the same experimental methods used to elicit oviposition digging. The activity, recorded extracellularly, was oviposition-like in burst period (5–6 s) and homologous muscle phase relationships, and it persisted after sensory inputs were removed, indicating the presence of pregenital CPGs. The abdomen exhibited posterior-going waves of activity with an intersegmental phase delay of approximately 1 s. These results indicate that serially homologous motor systems, including functional CPGs, provided the foundation for the evolution of oviposition behavior.

Keywords CPG · Evolution of behavior · Motor pattern · Insect abdomen · Oviposition

Abbreviations

3EL	3rd External Lateral
A1–A11	Abdominal segments 1–11
CI	Common Inhibitor
CLOSE	Closer
CPG	Central Pattern Generator
DUM	Dorsal Unpaired Median
EV	External Ventral
OPEN	Opener
Nv 1	Lateral Nerve 1
Nv 2	Lateral Nerve 2
PARA	Paradorsal
PRO	Protractor
RET	Retractor
SD	Standard Deviation
T1–3	Thoracic segments 1–3

Introduction

Central pattern generators (CPGs) are neural circuits underlying innate rhythmic animal behaviors such as breathing and locomotion (Marder and Rehm 2005; Guertin and Steuer 2009), and sexually dimorphic behaviors such as ejaculation, vocalization, and oviposition (McKenna and Nadehaft 1986; Thompson 1986a; Rhodes et al. 2007; Pavlou et al. 2016). Life-stage-specific behaviors, such as hatching and molting, are also served by CPGs (Truman 1980; Bekoff and Kauer 1984). For a small number of these circuits, the component neurons have been identified. However, for many others, they have not. Nevertheless, the existence of a CPG can be firmly established by recording a motor pattern similar to the natural behavior in the "fictive" condition. That is, in the absence of timing cues from either higher neural centers or sensory feedback, the isolated nervous system generates the rhythmic motor pattern which is recorded extracellularly from nerves and muscles.

Grasshoppers and locusts produce an idiosyncratic oviposition behavior in which distinctive appendages located on the female abdomen tip (Fig. 1a) dig a deep hole for egg deposition (see Fig. 1b). The oviposition digging CPG is located in the terminal abdominal ganglion of the female

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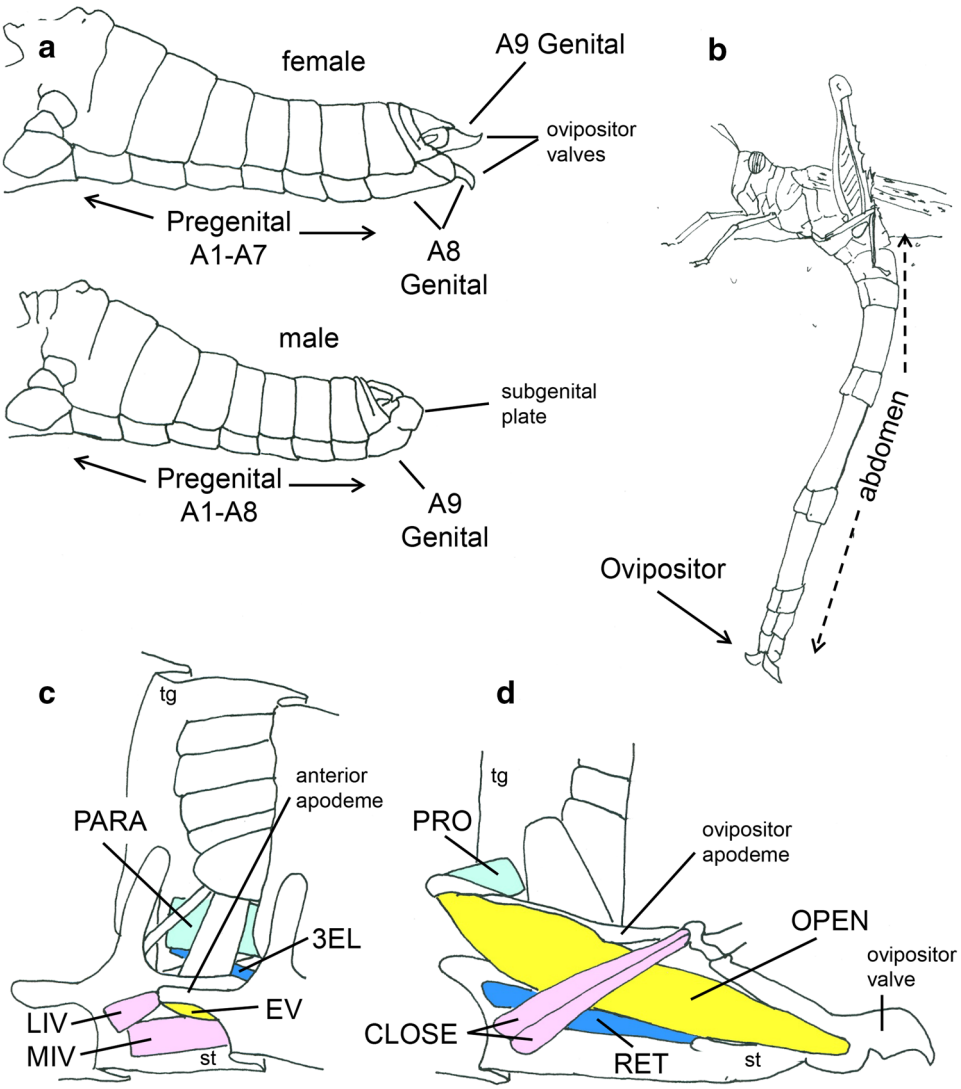

Fig. 1 Grasshopper abdomens, oviposition behavior, musculo-skeletal morphology of pregenital, and the female eighth abdominal segment (A8). **a** Female (upper) and male (lower) abdomens showing the segments of the pregenital and genital regions, lateral views, posterior to the right (A8 and A9 female; A9 male). Abdominal segments one through nine are abbreviated A1–A9. The elongated female A8 segment with attached ventral ovipositor valves contrasts with plain pregenital segments, A1–A7 in females and A1–A8 in males. The male phallic apparatus is located within the genital chamber and covered by the subgenital plate of segment A9. The female's A9 segment bears the second set of ovipositor valves dorsally on the abdomen tip (A9 genital). **b** Oviposition behavior. Rhythmic movements of the ovipositor valves have excavated a hole in the sand stretching the pregenital abdomen into the ground, approximately 10 cm, in this image redrawn from a photograph. **c** The morphology of body wall muscles on the right side of a typical pregenital abdominal segment, shown from an internal lateral view with posterior to the right. The small dilator and occlusor muscles of the spiracle are not shown, nor is a small oblique muscle, the lateral external dorsal muscle, found in the tergal fold. Muscles are located ventrally on the sternal plates of exoskeleton (st) and laterally on the tergites (tg). Intersegmental muscles attach to the cuticle of the next posterior segment, including the anterior apodeme. In d, the right ovipositor muscles are shown also from the internal lateral view. Pregenital and genital homologues are indicated by the same tone. Note the fusion of the pregenital MIV and LIV muscle homologues as the ovipositor CLOSE muscle, and the enormously enlarged, compared to the EV muscle, intersegmental OPEN muscle inserted onto the face of the extended ovipositor apodeme of the A9 segment. Sternum (st) and tergum (tg) are indicated, see Table 1 for further information on muscles (Thompson et al. 2014)

ventral nerve cord (Thompson 1986a). The oviposition CPG is activated by removal of tonic inhibition descending from the metathoracic ganglion to the terminal abdominal ganglion (Thompson 1986b; Leverett and Thompson 2011). The CPG can be experimentally activated at any time by transecting the nerve cord or applying a cold block of nerve conduction. The activity is readily recorded extracellularly and it occurs spontaneously for hours following the transection.

Experimental activation, by nerve cord transection below the metathoracic ganglion, has been used previously to investigate the oviposition CPG in other than sexually mature individuals. CPG activity was induced in immature animals, and recorded from developing ovipositor muscles in immature adults, larvae, and embryos dissected from eggs as young as 80% of embryonic development (Thompson and Roosevelt 1998).

During the initial phase of egg-laying behavior, the shovel-shaped ovipositor valves (Fig. 1a) swing open and closed about their hinges to dig a hole in the ground (Thompson 1986a). Numerous adaptations of female cuticle and intersegmental muscles allow for the enormous extension of the abdomen, e.g., 10 cm, that takes place as the ovipositor digs underground, stretching the abdomen after it (Jorgensen and Rice 1983; Vincent 1976). Complex sensory structures (Kalogianni 1996; Tousson and Hustert 2000; Wanischeck and Rose 2005; Newland and Yates 2008b) serve the behavior, and adult maturation of ovipositor skeleton and muscle have been characterized (Rose 2004; Thompson et al. 2014). Also present are neural circuits controlling coordinated oviduct and spermathecal contractions (Lange et al. 1984; DaSilva and; Lange 2011). Periodic pauses during excavation allow the abdomen to rotate and press against the walls of the hole, tamping to stabilize the sides. Ultimately, an egg pod is deposited deep underground, arranged, so that the heads of future embryos are pointed upwards for escape of the new hatchlings. The pod is capped by a frothy secretion that hardens as the abdomen is removed from the hole at the end of the process. The froth cap provides a pathway for escape of the hatchlings after their development underground, and protects them from desiccation and predation by birds. The deposition behavior occurs once each week to ten days in sexually mature adult females.

Grasshopper oviposition is unlike most other insects' egg-laying behavior in that it involves ovipositor appendages homologous with legs (Snodgrass 1935). In only two families of the orthopteran order of insects, the acridids (including grasshoppers) and the tettigonids, does the ovipositor develop from embryonic limb buds (Matsuda 1976). Embryonically, each grasshopper body segment carries a similar pair of serially homologous ventral appendage rudiments. These embryonic limb buds give rise to antennae and mouthparts in the head, legs in the thorax, and ovipositor valves in genital segments of female abdomens. By contrast, pregenital segments of the abdomen secondarily lose their appendages during embryonic development.

Males, obviously, do not produce oviposition behavior. They also do not have ovipositor appendages in their genital region, and the pregenital abdominal segments of both sexes are unadorned and without movable appendages (Fig. 1a). The resulting sexual and segmental diversification of behavior in adults occurs despite the segmentally uniform neuroblast arrays and serial appendages found in embryos, regardless of body region, sex, or specialization (Goodman and Bate 1981; Thomas et al. 1984; Jarvis et al. 2012). It is, therefore, unclear how, from a uniform beginning, the processes of development and evolution would produce a nervous system capable of supporting the grasshopper's unusual oviposition behavior. We considered two testable hypotheses as potential explanations. First, that the female genital nervous system would possess supplemental neuronal circuitry for oviposition behavior. Consistent with this hypothesis, the terminal abdominal ganglia of males and females are enlarged compared to pregenital ganglia. They are fused ganglia, appearing apple-shaped in females, broader at the rostral end where the eighth neuromere is located, than the corresponding pear-shaped ganglion of males. In addition, as a precedent, thoracic ganglia are significantly larger and contain many more neurons than abdominal ganglia. This difference in size is clearly related to the demands of controlling legs and wings, and the larger size of the terminal abdominal ganglion in females may be related to the demands of controlling the ovipositor. Second, that additional neurons were not added to the motor system, meaning that segmentally reiterated elements of the central nervous system must have been repurposed for oviposition. These two hypotheses generate different predictions that can be tested by comparative studies of pregenital and genital ganglia. In the first case (additional neurons for oviposition), the pregenital ganglia would be expected to be missing the oviposition elements. In the second case (repurposed neurons for oviposition), neurons and circuits corresponding to oviposition components of genital segments would be found in pregenital abdominal ganglia, including possibly those of males.

Our previous comparative morphological analysis of the pregenital and female genital abdomen worked through the complexity of the neuromuscular and skeletal systems, and we discovered widespread serial homologies of the ovipositor and pregenital structures (Thompson et al. 2014). Although the homologies were initially elusive, the precise correspondence of multiple complex morphological traits was convincing, leading us to now favor the second hypothesis. Specifically, the plain pregenital segments of adults hold 14 similar pairs of muscles per segment, occurring as thin sheets lining the body wall. The female's eighth abdominal segment (A8), which is one of the two genital segments, had been shown long ago to be missing five of the standard reiterated pregenital abdominal body wall muscles, but to contain four specialized ovipositor muscles not found in pregenital segments (Snodgrass 1935). We noticed, however, that the four ovipositor muscles in female segment A8 were each innervated by nerve branches corresponding to those supplying the five pregenital body wall muscles thought to be lost (Thompson et al. 2014). We backfilled these lateral nerve branches in pregenital ganglia to retrogradely label

efferent neurons for comparison with previously characterized ovipositor neurons. Based on multiple morphological criteria, our comprehensive study showed that ovipositor and pregenital efferent neural elements together comprised complete matching sets of serial homologues, and that the five "missing" muscles were actually present in the female's A8 segment, recast as four paired ovipositor muscles with similar innervation (Fig. 1c, d; Table 1). The muscle number disparity is resolved, because the two-headed ovipositor closer (CLOSE) muscle represents two fused pregenital muscles (MIV and LIV). Minor differences included two extra ovipositor opener (OPEN) motor neurons compared to the external ventral (EV) motor neurons, and two of the EV motor neurons displayed thick decussating neurites that were not found in the opener motor neurons, whose branches were all ipsilateral (Thompson et al. 2014).

Thus, while evolution has essentially rendered unrecognizable the serial homology of skeleton and muscles due to loss of appendages in the pregenital abdomen, homology has nevertheless been confirmed by correspondence of innervation and muscle attachments on the modified ovipositor exoskeleton. In fact, most ovipositor motor neurons, dorsal unpaired median (DUM) neurons, and common inhibitor (CI) neurons are essentially indistinguishable from their pregenital homologues in number, cell body position, primary neurite trajectory, and neurite branching pattern (see Thompson et al. 2014). As summarized in Table 1, seven ovipositor CLOSE motor neurons were found to be located in the A7 ganglion, the anterior adjacent ganglion to the terminal abdominal ganglion, along with CI neuron. The matching pregenital MIV and LIV muscles are supplied, respectively, by four and three motor neurons in the anterior adjacent ganglion in relative positions overlapping those of the CLOSE motor neuron cell bodies, and also a single shared CI neuron. A DUM neuron in the local ganglion was also found to supply the CLOSE muscle in A8. Similarly, a local DUM neuron was found to be shared by the MIV and LIV muscles in pregenital segments. The ovipositor PRO muscle is supplied by two contralateral motor neurons, as is the homologous pregenital PARA muscle. Each is also supplied by an anterior adjacent CI neuron and a local DUM neuron. The ovipositor OPEN motor neurons were located ipsilaterally in the ganglion adjacent to the root of Nv 2, as were the homologous pregenital EV motor neurons. Each is also innervated by a local DUM neuron, but no CI neuron. The two ovipositor RET motor neuron cell bodies are ipsilaterally anterior in the local ganglion as are the two homologous 3rd External Lateral (3EL) motor neurons. The RET and 3EL muscles similarly do not receive DUM or CI neuron innervation. The widespread homologies meant to us that it was unlikely the motor infrastructure underlying oviposition behavior required the evolution of new neural and muscular elements. Rather, it appeared that a repurposing and modification of reiterated abdominal structures had occurred in the genital segments.

A key question, however, had remained unanswered. Were there also serially homologous CPGs in the pregenital abdominal ganglia? In this study, the emphasis moved

Table 1 Summary of genital and pregenital neuromuscular homologies

Nerve	Female A8 Ovipositor				Male and female pregenital			
	Muscles	MNs	DUMs	CIs	Muscles	MNs	DUMs	CIs
Nv 1	CLOSE (closer, 247)	7	1	1	MIV (median int. vent, 172)	4	1	1
					LIV (lateral int. vent., 173)	3		
		Ant	Local	Ant		Ant	Local	Ant
	PRO (protractor, 256)	3	1	1	PARA (paradorsal, 169)	3	1	1
		Local	Local	Ant		Local	Local	Ant
Nv 2	OPEN (opener, 272)	7	1	0	EV (ext. vent, 174)	5	1	0
		Local	Local			Local	Local	
	RET (retractor, 248)	2	0	0	3EL (3rd ext. lat., 179)	2	0	0
		Local				Local		

The four ovipositor muscles of the eighth abdominal segment (A8) and their innervations in females are listed across from their male or female pregenital homologues as fully described in Thompson et al. (2014). Innervation by Nerve 1 (Nv1) or Nerve 2 (Nv2), ovipositor muscles in A8, the closer (CLOSE), protractor (PRO), opener (OPEN) and retractor (RET), and number and locations of motor neurons (MNs), dorsal unpaired median neurons (DUMs) and common inhibitor neurons (CIs) supplying them are indicated on the left half of the table. On the right, these elements are aligned with their morphological homologues in pregenital abdominal segments. The two-part CLOSE muscle and neurons align with the median and lateral internal ventral (LIV and MIV) muscles and neurons, the PRO with the paradorsal (PARA), the OPEN with the external ventral (EV) and the RET with the 3rd external lateral (3EL) muscles and neurons. Cell body position of neurons, in same ganglion as the lateral nerve, local ganglion (Local) or in the anterior adjacent ganglion (Ant.) is also indicated. Muscle nomenclature and numbers are after Snodgrass (1935) and Thompson (1986a). Abbreviations for pregenital muscle names- int: internal, ext: external, vent: ventral, lat: lateral

to nervous system *function*, complementing the previous morphological analysis, through a physiological comparison of genital and pregenital segments of the abdomen of the grasshopper, *Schistocerca americana*. Thus, the motor pattern-generating capacity of a specialized genital segment in females was compared to that of the plain unadorned and sexually monomorphic pregenital segments in both male and female grasshoppers. One of the two genital segments, A8, was chosen as the focus for comparison to pregenital segments. The other genital segment in females is A9 (Fig. 1a). Pregenital segments comprise segments A1–A7 in females and A1–A8 in males, since A9 alone bears the phallic apparatus, hidden in the genital chamber behind the subgenital plate (Fig. 1a). The appearance of segment A8 in females contrasts markedly with the plain pregenital segments. Externally, the heavily sclerotized left and right ovipositor valves are prominent as is the elongated sternal plate. Internally, the pronounced musculature, e.g., the enormously enlarged OPEN muscle, and large ovipositor apodeme obscure any similarity to pregenital segments (Fig. 1c, d). In addition, the A8 central nervous system is part of the terminal ganglionic mass which comprises A8–A11, while the pregenital ganglia we studied are unfused (see Fig. 2a).

We did not know if pregenital CPGs could be experimentally activated, nor had we identified any component interneurons of the oviposition digging CPG in the female's terminal abdominal ganglion to use for comparison. It was possible that even if pregenital CPGs were present, they could be resistant to activation and thus not observable physiologically. Furthermore, another possibility was that pregenital CPGs may have been lost during development concomitant to the loss of pregenital abdominal appendages. Despite these potential problems, in the present study, nerve cord transections were combined with extracellular nerve and muscle recordings to successfully elicit, assess, and characterize the motor patterns of pregenital abdominal CPGs in male and female grasshoppers. The results have implications favoring a certain pathway for evolution of the specialized female, life-stage-specific, CPG-based behavior. For reviews of other comparative analyses focused on the problem of neural circuit evolution for behavior, see Arbas et al. (1991), Guertin and Steuer (2009) and Katz (2016). Aspects of rhythmic abdominal ventilatory behavior and induced movements of the male phallic apparatus were also considered.

Materials and methods

Adult male and female grasshoppers, *Schistocerca americana*, obtained from our own laboratory colony were used for this study. Anatomical nomenclature is based on Snodgrass (1935), Seabrooke (1968), and Thompson (1986a). Animals were dissected by decapitation followed by removal of the jumping legs and wings. Specimens were secured in paraffin wax-filled dishes ventral side down using dental wax, and a

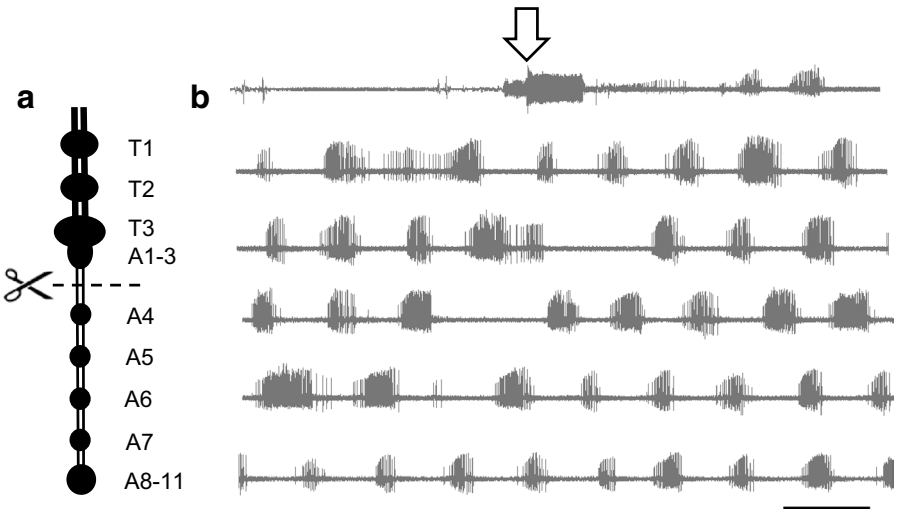

Fig. 2 Experimental activation of rhythmic pregenital bursting activity. Bursting activity was induced by transection of the ventral nerve cord behind the metathoracic ganglionic mass as indicated in (**a**). In this diagram, T1–3 refer to the thoracic ganglia or neuromeres, and A1–11 refer to abdominal ganglia or neuromeres. The metathoracic ganglionic mass, or metathoracic ganglion, is composed of the T3 and first three abdominal neuromeres A1–A3. The terminal abdominal ganglion consists of neuromeres A8–A11. In **b**, which is a continuous electromyographic recording from a MIV muscle in a male's A6 segment, the muscle was silent at the beginning of the recording before nerve cord transection. At the time indicated by the arrow, the nerve cord was transected. Transection induced bursts of muscle activity which soon became rhythmic. In preparations such as this, the rhythmic motor pattern would continue spontaneously for hours. Scale bar: 5 s

mid-dorsal incision was made through the cuticle the length of the abdomen. The abdomen was pinned open, keeping the left and right tergal plates vertical so as to form a container for saline. The gut and gonads were then removed to reveal the ventral diaphragm and underlying ventral nerve cord, lateral nerves and muscles of interest. Preparations were kept moist with physiological saline.

Electromyographic recordings of abdominal muscles, either singly or two at a time, were made with suction or silver wire electrodes placed directly onto the specific muscles under saline. Silver hook electrodes, insulated with petroleum jelly, were used to record from lateral nerves. Signals were amplified with A–M Systems Differential AC Amplifiers (Model 1700, Sequim, WA, USA) and transcribed through a DATAQ 720 data acquisition system (Akron, OH, USA). For these studies of pregenital motor patterns, 77 male and 42 female animals were used. To calculate burst period, data were analyzed by measuring cycles from the start of one burst to the start of the next burst. Statistical data were presented as mean ± standard deviation, SD, using 50 cycles per animal. For the majority of recordings from pregenital segments, a ventral longitudinal muscle, the median internal ventral (MIV) muscle, was used. The MIV muscle is the largest and most accessible of the pregenital ovipositor muscle homologues. It is homologous to the median head of the ovipositor closer (CLOSE) muscle (Fig. 1c, d; Table 1).

Results

Grasshopper abdomens are visibly segmented with a larger, pregenital region, segments A1–A7 in females (A1–A8 in males), and a sexually modified terminal region (Fig. 1a). The 14 pairs of reiterated pregenital abdominal muscles are located superficially in the abdomen (Fig. 1c). The majority of the intersegmental muscles produce retraction, or shortening of the abdomen; only a single muscle pair, the external ventral (EV) muscles, directly produce abdominal extension. Three muscles are dorso-ventral, involved in ventilation, while two other intra-segmental muscle pairs are oblique and cause twisting or curving movements (Snodgrass 1935; Hustert 1974). Among the body wall muscles and found ventrally or ventro-laterally in pregenital segments are the five pregenital homologues of ovipositor muscles (Fig. 1c, d; Table 1; Thompson et al. 2014). Most of these muscles are thin and strap-shaped and all, except the third external lateral muscle (3EL), are intersegmental, originating in the local segment and inserting into the posterior adjacent segment. In addition to the 3EL muscle, the median internal (MIV) muscle, lateral interior ventral (LIV) muscle, and paradorsal (PARA) muscle are retractors. The EV muscle produces protraction.

In females, the two genital segments, A8 and A9, generate digging movements in oviposition (Fig. 1a, b), whereas in males, there is only one genital segment, A9 (Fig. 1a). (Segments A10 and A11 are considered post-genital in both sexes.) Pregenital abdominal segments appear sexually monomorphic, except that the muscles of the smaller male are proportionally thicker than those of females. Studies of the ovipositor muscles of A8 (Fig. 1d) showed origins within the segmental plates of segment A8, and insertions on modified segment A9 cuticle specialized to include enormously enlarged anterior sternal apodemes, now called the ovipositor apodemes, and a much reduced A9 sternal plate, where muscle insertions converge medially (Snodgrass 1935; Thompson et al. 2014).

Elicited pregenital motor pattern

To assay the functional capacity of pregenital ovipositor homologues, the ventral nerve cord was cut rostrally in the abdomen behind the metathoracic ganglionic mass. Cutting the nerve cord in this location, between A3 and A4 (Fig. 2a), induced a rhythmic posterior-going wave-like movement along the length of the abdomen in which contractions of pregenital muscles including the MIV preceded homologous ovipositor muscles. The transection also led to a wave-like movement along the abdomen in male preparations, whereby here the phallus was rhythmically active in the terminal segment and coupled to pregenital body wall movements. In both males and females, before transection and similar to oviposition recordings, pregenital MIV muscles were inactive. Transection was found to reliably induced oviposition digging activity in females (Thompson 1986b; Leverett and Thompson 2011). The method likewise readily activated rhythmic bursting in pregenital abdominal segments of both sexes, as shown in the example of an electromyographic recording from a male pregenital MIV muscle in segment A6 (Fig. 2b). Immediate intense activity in the MIV muscle was induced by the cut, and simultaneously in the other MIV muscles along the entire abdomen (not shown). The whole abdomen shrug was followed, usually within 10 s., by the establishment of a rhythmic pattern of bursting. The pregenital bursting activity was robust and it continued to occur spontaneously for hours. Similar to oviposition, no exogenous pharmacological agents were needed to sustain hours of vigorous bursting. Remarkably, in scores of animals tested in these and other studies, transection activated the pregenital motor pattern without fail, 100% of the time. Mean cycle period was 5.82 ± 0.2 s SD, $N = 10$ animals, range of mean cycle period was (4.75–6.52 s) also similar to recordings of the oviposition digging motor pattern. No differences were observed between recordings of males (mean period 5.77 ± 0.3 s) and females (mean period 5.89 ± 0.18 s).

The MIV muscle bursting activity was accompanied by bursts in the four remaining homologous pregenital muscles (Fig. 3). Pairwise electromyographic recordings from pregenital muscles within individual segments were obtained using the MIV muscle recording as a common reference. The phase relationships of the complete homologous set were examined. Recordings revealed a simple motor pattern consisting of two phases of alternating bursts (Fig. 4a). In the pairwise electromyographic recordings of Fig. 3a–d, MIV recordings are in the top traces. The lower trace is from the indicated muscle ipsilateral in the same segment. In Fig. 3a, b, the recordings reveal that the EV muscle and the PARA muscle are active in alternation with the simultaneously recorded MIV muscle. Conversely, in Fig. 3c, d, the 3EL and LIV muscles are coactive with the MIV bursting. The co-active EV and PARA bursts alternated with co-active MIV/LIV and 3EL bursts. Thus, the recordings displayed a motor pattern that matched the corresponding female A8 muscles (Fig. 4b) expressing the oviposition digging motor pattern (Thompson 1986a). Both motor patterns are bilaterally synchronous (Fig. 4c). The pattern of MIV and/or LIV bursts alternating with EV and PARA bursting directly parallels the oviposition motor pattern, where the homologous ovipositor CLOSE muscle bursts alternate

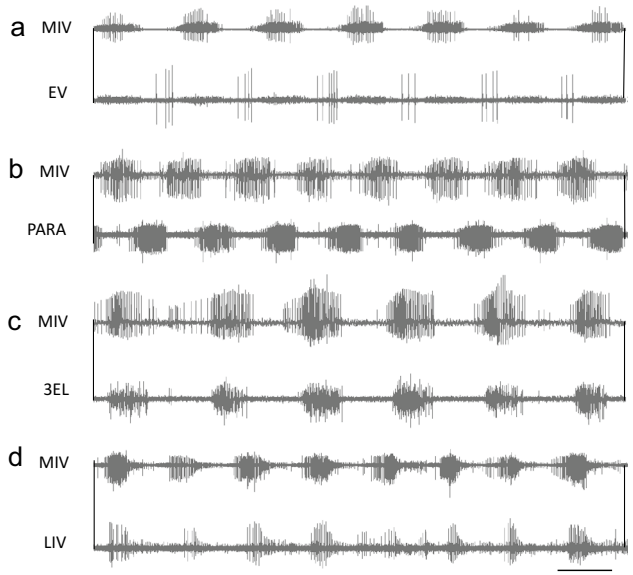

Fig. 3 Pregenital motor program. Simultaneous electromyographic recordings of pairs of pregenital muscles during production of the induced motor program after transection. In all records, the upper trace is MIV muscle activity. Recordings of MIV and EV muscles (**a**), and MIV and PARA muscles (**b**) revealed a rhythmic pattern of alternating bursts. In contrast, the MIV and 3EL muscles (**c**), and the MIV and LIV muscles (**d**) produced coactive bursting. Small units in the EV recording of part (a) are crosstalk from MIV bursting. Scale bar: 5 s

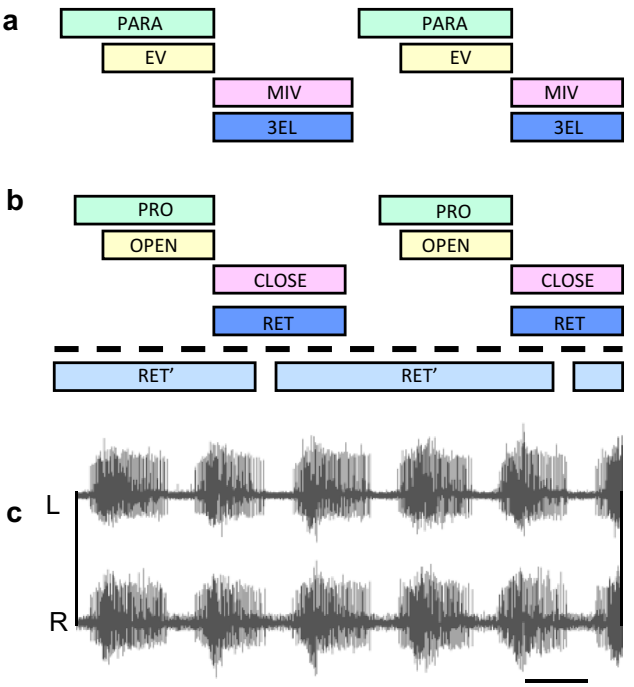

Fig. 4 Similarity of the elicited pregenital motor pattern to the female oviposition digging motor pattern. Phase diagrams of the pregenital motor program (**a**), and the oviposition digging motor program after Thompson (1986a) (**b**). A simple two-phase motor program is observed. The homologous muscles of the ovipositor CLOSE muscle, pregenital MIV/LIV muscles and of the ovipositor RET muscle, pregenital 3EL muscles, burst alternately with the homologues of the OPEN and PRO muscles, the EV and PARA muscles. In oviposition, closer and retractor muscles burst alternately with the bursts of opener and protractor muscles. RET' indicates an alternative form of the oviposition pattern that was also observed. Bars indicate burst activity (average durations, combined data from 12 males and 10 females, 50 cycles each) with genital and pregenital muscle homologues indicated by the same tone. In **c**, bilateral synchrony of the pregenital muscles is shown, as in this simultaneous recording of MIV muscles in a female's A5 segment. Scale bar: 5 s

with the ovipositor OPEN and PRO muscle bursts (Fig. 4a). No differences were found between the phase relationships of pregenital motor patterns expressed by female or male grasshoppers, nor across abdominal segments. The usual timing of ovipositor RET muscle activity recorded in the previous oviposition studies (Thompson 1986a) was co-activation with their antagonists, the PRO muscles, and their bursts were also prolonged with only a brief silence during CLOSE activity (Fig. 4b, see RET'). The 3EL burst pattern in pregenital segments was simpler, the bursts alternating, rather than coactive, with the PARA bursts. They also were coactive with the MIV/LIV bursts. In numerous recordings of pregenital segments, prolonged 3EL activity was never observed. An alternate motor pattern in oviposition was seen in up to 25% of oviposition preparations, in which antiphasic activity of ovipositor RET and PRO muscles occurred,

similar to the pregenital pattern. This simpler oviposition motor program was particularly evident in the fictive (isolated nervous system) condition (Thompson 1986a; Newland and Yates 2008a). It, therefore, seems that a pattern comparable to the simpler of the two oviposition motor patterns matched the pattern induced in pregenital segments. Thus, in the pregenital motor pattern, CLOSE and RET homologues alternated their bursting activity with OPEN and PRO homologues, i.e., MIV/LIV and 3EL alternated with EV and PARA to produce the oviposition-like motor pattern (Figs. 3, 4).

When burst structures were compared, the induced oviposition digging pattern recorded from isolated abdomens appeared more precise than the pregenital motor pattern. During expression of the oviposition pattern (Thompson 1986a), the onset of CLOSE muscle activity coincided with abrupt cessations of OPEN muscle bursting and a delay occurred between the end of CLOSE muscle bursts before the OPEN muscle bursts began again. The recordings of CLOSE muscles were characterized by relatively small units firing at high frequency, while, in contrast, OPEN muscle bursts displayed some very large units. In addition, during motor pattern production, the PRO muscles became active during the delay before the OPEN bursts began, but the PRO muscle bursts also terminated abruptly at the same time as the OPEN muscle bursts. The structure of the motor program with staggered onsets of bursting, and sharp terminations of OPEN and PRO bursts was prominent in recordings of the oviposition digging motor pattern (Thompson 1986a, Fig. 4b). Qualitatively, by comparison, the pregenital motor program displayed less abrupt and strict onsets and terminations of bursting. Rather, the pregenital segments produced more variable burst structures, with most bursts characterized by increasing then decreasing spike frequencies during the burst. In many recordings from the MIV, low frequency discharges continued to occur between bursts resulting in burst overlap (e.g., Fig. 8b), and variable levels of motor unit recruitment were seen, with some bursts completely missing large units (compare Figs. 3a, 5b).

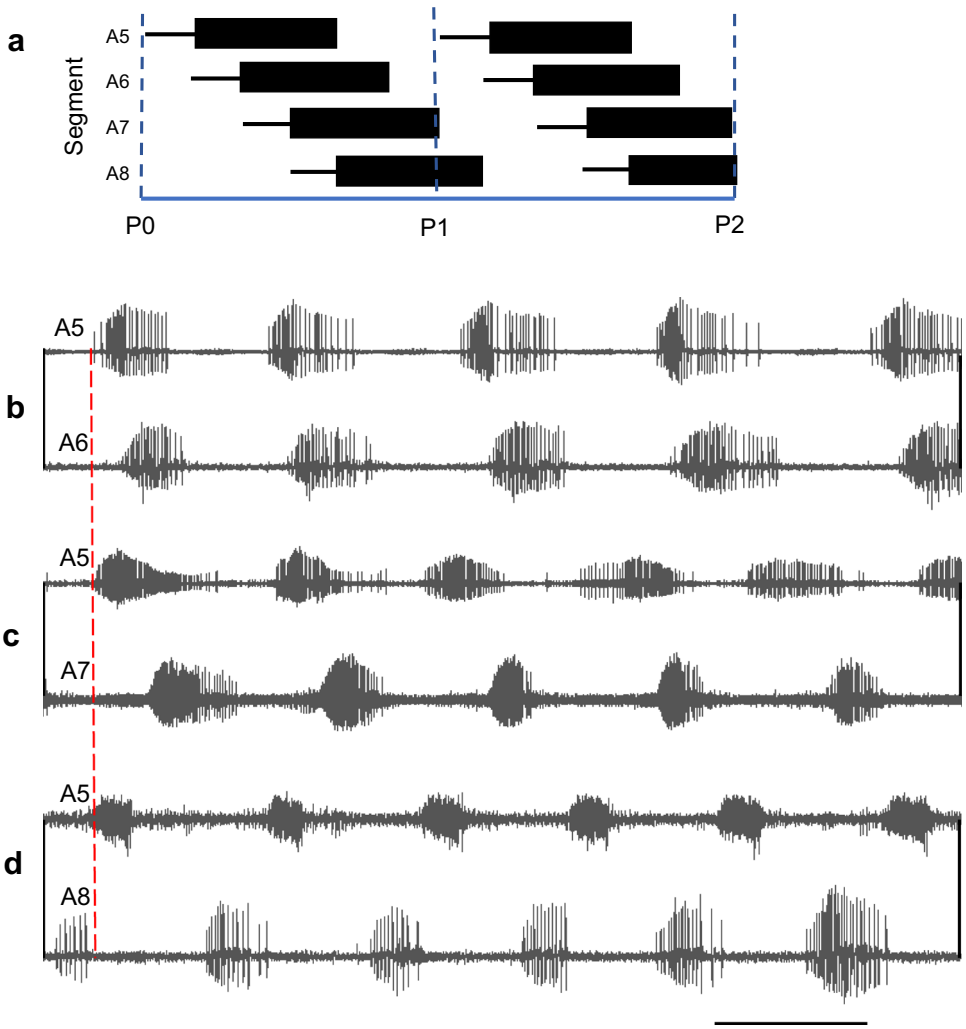

Fig. 5 Motor program occurred in waves of activity progressing posteriorly down the abdomen. Intersegmental phase relationships were determined from pairwise recordings of MIV muscles in different segments. **a** is a summary diagram of normalized MIV muscle activity as black bars with SD error bars for two full cycles of segment A5 activity and the coordinated bursts in segments A5–8. PO refers to the beginning of the cycle, P1 and P2 to the first and second periods of activity. Pairwise recording of two MIV muscles in adjacent segments (**b**), two segments apart (**c**), and three segments apart (**d**). The dashed vertical line serves to align the MIV muscle bursts in A5 across the separate records. Scale bar: 5 s

The pregenital pattern of rhythmic muscle bursts just described occurred in a pattern of recurring posterior-going waves that displayed intersegmental coordination along the length of the abdomen (Fig. 5a). The resulting movements consisted of minute alternating protraction and retraction of adjacent segments, one at a time, progressing caudally down the abdomen. Simultaneous recordings across abdominal segments revealed loose coupling and intersegmental phase lags of 0.95 ± 0.17 s SD (Fig. 5b–d). In preparations expressing a typical period of 6 s, the normalized mean phase delay between adjacent segments was 16% of cycle duration ($N=5$ animals). Measurements ranged from 14.2–23.1% of burst period. Thus, when recordings were separated by two segments, e.g., segments A5 and A7, the phase lag was approximately 32% (1.92 s ± 0.32 s SD, $N=5$ animals), and across three segments, the delay varied the most, but mean delay was calculated to be 48% ± 6.7 s SD ($N=5$ animals). Nerve cord transection in females, as previously demonstrated, also activated the oviposition digging motor program. Bursts in pregenital MIV muscles were similarly coordinated, as shown in Fig. 8, the A7 MIV muscles bursts preceded the homologous ovipositor CLOSE muscle bursts segment A8 (Fig. 8b). The wave progresses 5–6 segments caudally before beginning again (Fig. 5a).

Evidence for oviposition-like pregenital central pattern generators: fictive activity

Key tests for the presence of CPGs focus on their autonomy. The genital nervous system of females, specifically the terminal abdominal ganglion, has been shown to possess a CPG for oviposition digging. Isolated terminal abdominal ganglia removed from the animal, devoid of both higher center input and sensory feedback, produced fictive oviposition digging activity (Thompson 1986a, b). Also satisfying one criterion for demonstrating a CPG, the pregenital motor pattern was elicited by nerve cord transection, in which all neural inputs from higher centers were removed (Fig. 2). Then, to test for autonomous activity of the pregenital motor pattern, ganglia were isolated from posterior ganglia by cutting the posterior connectives and from sensory feedback by cutting all lateral nerves. Fictive bursting was recorded with hook electrodes from the posterior branch of Nv 1 that carries the MIV axons, proximal to the cut (Fig. 6a, b). In these recordings, two abdominal ganglia were together because the cell bodies of the MIV are located in the anterior adjacent ganglion to the ganglion from which their axons emerge. The isolated ganglia pair continued to produce rhythmic bursting discharges, but at a slower rate typical of fictive preparations, with a period of 8.75 ± 0.23 s SD $N=5$ animals (Fig. 6a, b). The regularity of the fictive bursting activity can be seen most clearly in Fig. 6b, a continuous recording of almost 7 min duration, in which the time base was slowed twofold. Burst durations were within the normal range and intensity. Individual isolated ganglia also were capable of fictive bursting activity, as shown in the EV recording from the posterior branch of Nv 2 of a single, isolated pregenital ganglion (Fig. 6c).

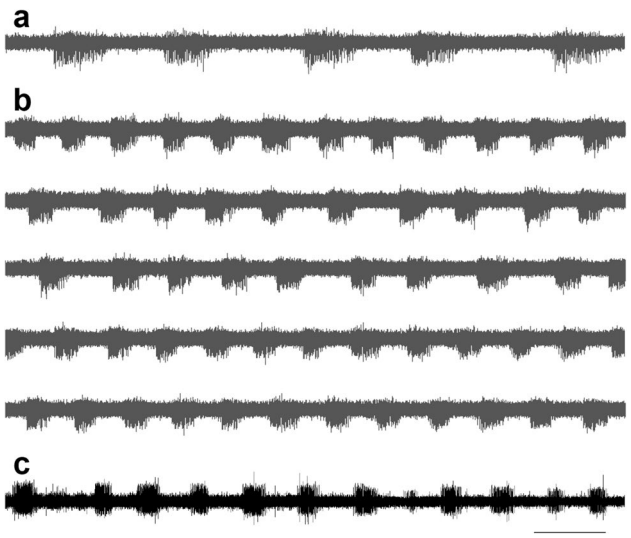

Fig. 6 Fictive pregenital motor pattern. Extracellular recordings of spontaneous activity in the isolated nervous system. In a and b, spontaneous activity was recorded from the first posterior branch of Nv 1 proximal to the cut lateral nerve in ganglion A6 of a female (**a, b**). This branch carries the motor neuron axons that supply the MIV/LIV muscles. The in vitro preparation consisted of an A5 and A6 ganglion and lateral nerve stumps; two ganglia were necessary, because the cell bodies of the MIV motor neurons are located in A5. The record in (**a**) is presented with a same time scale the same as other recordings in this paper. In **b**, the time is compressed for this continuous recording of nearly seven minutes of regular bursting activity. In **c**, the preparation consisted of a single isolated ganglion A6, and activity of the EV motor neurons was recorded from a branch of Nv 2. Scale bar (**a**): 5 s, scale bars (**b, c**): 10 s

Ventilation

Grasshoppers ventilate their tracheal respiratory system by rhythmic dorso-ventral movements of the abdomen (Miller 1960; Lewis et al. 1973; Hustert 1974). In most abdominal segments, the pumping action is produced by three tergosternal muscles pairs: two that produce exhalation and one that drives inhalation. These muscles, the first and second internal laterals (exhalation) and the first external lateral (inhalation) are present in each abdominal segment, except for the A8 segment of both sexes, and in this segment, the first internal laterals are missing, leaving still one expiratory and one inspiratory muscle (Snodgrass 1935). The first and second internal lateral muscles in exhalation compress the abdomen by raising the sternum in their segment, while the external lateral muscles lower the sternum, expanding the internal volume thus causing inhalation. Dorso-ventral

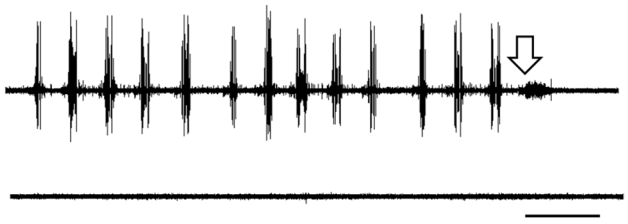

Fig. 7 Nerve cord transection abolishes the ventilatory motor pattern. In the dissected animal, ongoing ventilation was intense, as shown in this continuous recording of spontaneous activity in the recorded as short rhythmic bursts with a period of between 2 and 3 s from the first EL muscle, a muscle of inspiration. As soon as the nerve cord was cut behind the metathoracic mass (time indicated by the arrow), the rhythmic ventilation activity, with its burst period of between 2 and 3 s, immediately stopped and did not resume. Scale bar: 5 s

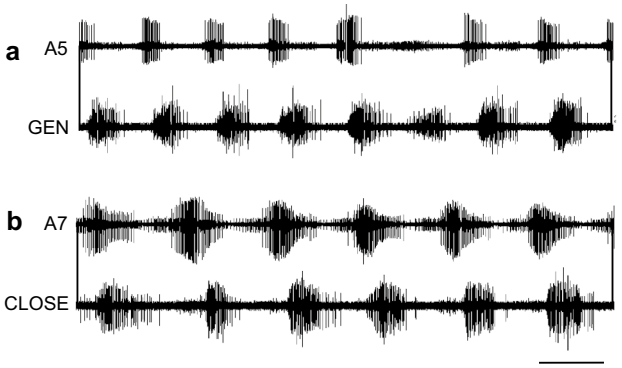

Fig. 8 Coordination between the pregenital and genital motor patterns in males and females. Electromyographic recordings of spontaneous activity after nerve cord transection. **a** Simultaneous electromyographic recordings from a male pregenital MIV muscle in segment A5 and the retractor of the phallus muscle (M261) of genital segment A9. The male motor program, similar to the oviposition digging motor program, was continuous and robust. **b** Pregenital MIV muscle in segment A7 of a female recorded simultaneously with the ovipositor CLOSE muscle in A8. Scale bar: 5 s

movements in ventilation are nearly synchronous across abdominal segments, having an intersegmental phase lag of less than 100 ms (Hustert 1975). The ventilatory movements and muscles involved are distinct from the actions and pregenital muscles studied here which essentially produce small lengthwise telescoping movements of the abdomen descending in a slow travelling wave down the body axis. They display a 10X longer intersegmental phase lag, and substantially longer period. We routinely decapitated the grasshoppers before our experiments (see methods). In these preparations, ventilation was continuous, intense, and regular with a burst period of 1–2 s (Fig. 7), much faster than oviposition digging or the pregenital pattern examined here, with their burst periods of about 6 s. In addition, in decapitated grasshoppers, the oviposition pattern was not expressed, after the induction of a few spurious bursts (Thompson 1986b), nor was the pregenital oviposition-like motor pattern expressed. However, once the connectives were cut between the thorax and abdomen, the ventilatory pattern immediately ceased and did not resume (Fig. 7), while, in contrast, the recording of the oviposition-like pregenital motor pattern showed that once initiated it persisted (Fig. 2).

Activation of a genital motor pattern in males

The transection used in this study also activated a rhythmic motor pattern in muscles of the phallic apparatus of males. Male bursting activity, expressed by genital muscles in segment A9, was recorded numerous times in various phallic muscles. As found in the recordings from females, where ovipositor muscle contractions were coordinated with pregenital muscle activity (Fig. 8b), in males the bursting activity recorded from the retractor muscle of the phallus (M261) was tightly coordinated with the pregenital motor pattern (Fig. 8a). The intersegmental functional relationships are clear, emphasized in the recording shown, where a rare missed burst in the pregenital MIV (upper trace) preceded a significantly weaker burst in the male genital muscle.

Discussion

Determining the evolutionary history of neural circuits for behavior is a challenging problem in neuroethology, but an important one because evolution is the unifying theme of the biological sciences (Arbas et al. 1991; Edwards and Palka 1991; Katz and Harris-Warrick 1999; Katz 2007, 2016). The comparative process provides key insights into the neural basis of behavioral diversity in animals. In the spirit of taking an evolutionary perspective, the present study is a comparative analysis of serial segments within the animal that are differently specialized for behavior. The objective was to gain insight into the evolutionary basis of oviposition by comparing genital segments with pregenital (non-ovipositing) segments. The finding of full rhythm-generating capacity and homology in pregenital ganglia indicates that massive central nervous system divergence was not required for evolution of oviposition neural circuits in the terminal abdominal ganglion of females. In contrast, extensive adaptation did take place in the morphological specializations of female exoskeleton, muscles, and sensory organs. Striking similarities are found in nervous system morphology of motor neurons, DUMs, and CIs between pregenital and genital segments. Lateral nerve organization and the entire populations of efferent neurons are nearly identical in all abdominal ganglia (Thompson et al. 1999, 2014). The

serially homologous ovipositor and pregenital neurons are a subset of these larger segmentally reiterated populations. Furthermore, from segmental limb buds to sternal apodemes and muscles, homology between pregenital segments and the female genital segment A8 was found to be the rule. Each structure in the oviposition motor system is aligned to either an embryonic or adult component of pregenital segments. The present study reveals, additionally, that nervous system homologies extended deeper into the central nervous system, to the interneurons, and importantly to the homologous CPGs. Oviposition-like pregenital motor patterns were experimentally induced by the same methods that activated oviposition for laboratory study. Similar to oviposition, the pregenital pattern, once elicited, was continuously active for hours. Fictive pregenital motor pattern activity was also observed, establishing the existence of oviposition-like pregenital CPGs in males and females.

Pregenital central pattern generators and evolution of oviposition

The evidence of functional motor output driven by serial abdominal CPGs extends and complements the previous finding of serially homologous efferent neurons in the pregenital and oviposition motor systems (Thompson et al. 1999, 2014). Together these results point to a remarkably conservative evolutionary foundation for the grasshopper's specialized oviposition behavior. Homologous neural components in pregenital and genital segments appear to mediate different behavior because of segmental differences in peripheral morphology. Skeletal adaptations for oviposition include the selective retention and elaboration of limb rudiments of female genital segments into complex ovipositor valves, enlargement of the ovipositor apodemes, and reduction of the sternal plate in A9 to a small midline structure. Two muscles changed their insertions, as the ovipositor PRO muscle now inserts on the tip of the ovipositor apodeme instead of on the anterior margin of the posterior adjacent segment's tergum (where the pregenital PARA muscles insert). The ovipositor RET muscle inserts posteriorly on the A8 sternum near the base of the valves instead of the lateral insertion on the local tergum of the pregenital 3EL muscles. Also, the OPEN muscle displays hypertrophy while the CLOSE muscle's two heads corresponding to the fused pregenital MIV and LIV muscles still insert on the sternal plate of the posterior adjacent segment, but the plate is tiny in segment A9. The neuronal building blocks for oviposition in segment A8, including the individual motor neurons, DUMs, CIs and CPG circuits, appear to be equivalent to modules segmentally reiterated throughout the pregenital abdomen, and they are not different in males. There is no evidence that oviposition results from added, unique neuronal circuitry. The larger size of the rostral region of the female terminal abdominal ganglion is, therefore, likely due to the additional demands of sensory processing of specialized inputs from tactile and chemoreceptive hairs and the substantial proprioceptive organs associated with oviposition. This suggestion is consistent with the finding that DUM interneurons, known to be receptive to sensory inputs in other segments, are more numerous in female A8 segment than in male A8 or pregenital ganglia (Thompson and Roosevelt 1998).

Conservative central nervous system

Further evidence of the conservative nature of the insect nervous system is observed in insect embryos. From the more primitive silverfish to the advanced holometabolous insects such as *Drosophila*, the embryos of insects of all orders display a common plan for neurodevelopment. Head, thoracic and abdominal neuromeres contain virtually identical arrays of segmental neuroblasts (Thomas et al. 1984; Stollewerk and Simpson 2005; Technau et al. 2005). Accompanying this reiterated nervous system morphology is the serially homologous paired ventral appendage rudiments of each body segment. These transient limb buds in abdominal segments are essential elements for the developmental process of peripheral nerve pathfinding through the actions of guidepost cells and limb-related afferent neurons (Meier et al. 1991). Modern insect appendages are considered to be serially homologous structures that retain anatomical and developmental aspects of their common evolutionary origin (Matsuda 1976; Boxhall 2004; Hoch et al. 2004; Bowsher and Nijhout 2009). An additional feature of evolution in insects is that limbs has been suppressed from the abdomen in a majority of insects. Abdominal appendages are suppressed by the Hox genes Ultrabithorax (Ubx) and abdominal-A (abd-A), reviewed in Hughes and Kaufman (2002).

Comparative studies in other systems have repeatedly shown that nervous systems tend to be more conservative than the periphery (Dumont and Robertson 1986; Arbas et al. 1991; Edwards and Palka 1991; Edwards 1997; Guyenet 2006; Guertin and Steuer 2009). Similar neuronal circuitry has been found to underlie divergent behavior in crustaceans. In decapod crustaceans for example, Paul (1989) discovered that homologous neural circuits drove diverse behaviors in tail fans largely because of peripheral differences of homologous muscular insertions. In an important examination of the nervous system in an insect that lost flight behavior in evolution, Arbas (1983a, b) found flight reflexes and elements of the flight circuitry retained in the nervous system of flightless grasshoppers. The common ancestor of modern insects had wings and flew. It is entirely possible that the neurons utilized in flight may have other roles in the development or biology of flightless insects. In this regard, it is interesting to note that some neurons of locusts display

both flight and respiratory activity (Burrows 1975; Dumont and Robertson 1986).

Intracellular studies of mollusks have produced further understanding of diversification of neural circuits during evolution. Experiments with identified neurons in the swim circuits of nudibranch mollusks (Newcomb and Katz 2007, 2009) have revealed that two species may have homologous neurons, but in one species, the neuron is a component of the swim CPG and the other it is not, serving instead as a modulator. In addition, divergent CPG circuits in different species can generate equivalent modes of swimming, or homologous behavior (Katz 2016). The studies of arthropods reveal that peripheral modifications can lead to behavioral differences without necessarily requiring alterations of neural circuits. It is likely that the arthropods, with their versatile articulated appendages, could produce widely divergent behavior through peripheral adaptations alone. Articulated appendages in insects range from specialized mouthparts and antennae to claws, legs, abdominal appendages, cerci and stings. These adaptable appendages are associated with species-specific modes of locomotion, as well as with a variety of specialized behaviors. It can be argued that the success of arthropods is largely due to this versatility of their appendages (Angelini and Kaufman 2005). Nudibranchs may be more reliant upon central nervous system variation to drive divergent movements through the peripheral nerve net to their muscles used for swimming. However, comparative analysis of feeding circuits between the opisthobranch *Aplysia* and the pulmonate snail indicate that divergent behavior may result from alteration in the peripheral innervation patterns of possibly homologous neurons (Wentzel et al. 2009). In addition, programmed cell death and hormonal influences provide additional mechanisms for adaptation and diversification of neural circuits in development and in metamorphosis (Tissot and Stocker 2000; Vasilakos et al. 2005; Buss et al. 2006). CPGs themselves are subject to hormonal and neuromodulatory influences that produce variations in circuit performance and configuration, underscoring the flexibility possible in structure and function of CPGs (Truman 1980; Ewer et al. 1997; LeFeuve et al. 1999; Fenelon et al. 2003; Marder and Rehm 2005; Zitnan et al. 2007; Marder 2012; Marder et al. 2015).

Comparative neuroethological studies of central pattern generation in vertebrates, the other major group of segmented animals, provides further insight. Lower vertebrates, such as lampreys and frogs, have been compared to rodents both with regard to respiratory circuitry in the brainstem and spinal control of locomotion. Homologous neurons were detected in lamprey swim circuitry and mammalian walking circuits. The walking circuitry was found to be axially distributed, similar to spinal locomotor circuit organization in fish swimming (Kiehn and Kjaerulff 1998). Homologous neurons and segmentally distributed modules in brainstem were also detected in the pattern-generating circuitries of water-breathing lampreys, frogs and air-breathing mammals (Vasilakos et al. 2005; Cinelli et al. 2013). Thus, the flexibility and modifiability of CPGs need to be kept in mind to avoid oversimplified interpretations. Likewise, it is clear that the co-opting of ancestral circuits for new forms of behavior is a recurring theme in comparative studies.

Pregenital central pattern generators and other rhythmic abdominal behaviors

The presence of reiterated oviposition-like CPGs in the pregenital grasshopper abdomen raises the question of their potential role in the animal's behavior. Because the CPGs are normally held silent under the control of descending neural inhibition, it is conceivable that pregenital circuits may be latent and never normally disinhibited for behavior. Other investigators have suggested that inhibitory control may constitute a nervous system strategy for managing circuits for behavior discarded in development, as opposed to dismantling them, as shown, for example, by the elicitation of "hatching" motor patterns in adult birds (Bekoff and Kauer 1984). In the insect *Manduca sexta*, larval-like ecdysis activity was elicited in adults by removing descending input from the pterothoracic ganglion (Mesce and Truman 1988). It is conceivable that the oviposition-like motor program represents the activation of latent molting CPGs in adult grasshoppers. However, while it is known that molting is a CPG-based behavior, its abdominal segmental activity is coordinated in a rear-to-front metachronal wave, whereas the oviposition-like pattern is front-to-rear. A rear to front pattern also characterizes the behavior of vermiform grasshopper larvae. Waves of abdominal movements are used by larvae to dig up to the surface after hatching. At room temperature, they have a period of 4.5 s in *Schistocerca gregaria* (Bernays 1971).

The physiological results demonstrate that the abdominal CPGs, including the oviposition CPG, are continuously active in the absence of tonic descending inhibitory input. Descending inhibitory influence is likely necessary for suppression of their activity which, due to membrane and circuit properties of the CPGs, would otherwise continuously drive costly rhythmic muscle contractions in the abdomen. Inhibitory control may have evolved as a necessary mechanism to govern the oviposition digging CPG (and the pregenital CPGs) because of inherent CPG excitability. In fact, the physiological properties of such a spontaneously active circuit may preclude excitatory control. The presence of pregenital CPGs in males argues against the notion that the pregenital circuitry's sole purpose is to contribute to oviposition behavior. The coordinated rhythmic motor pattern expressed by the genitalia of segment A9 in males also indicates that within the complex anatomy of the phallic apparatus, there

may be another story of serial homology with pregenital segments or of sexual homology with females.

Primordial abdominal CPGs may, therefore, constitute a multi-segment arrangement that formed the basis for evolution, within the genital segments, of specialized motor patterns related to reproductive behavior. Despite the altered intersegmental coordination, the experimental nerve cord transections could still be activating undismantled molting CPG circuits in pregenital segments. The molting oscillator may have been modified for reproductive behavior in genital segments during nervous system evolution separately from the intersegmental coordinating system, or it may be modulated or modified by juvenile hormone exposure during adult sexual development. A variety of developmental and chemical mechanisms could be involved with changing intersegmental coordination. Alternatively, an intriguing possibility given that the ovipositor valves develop from embryonic legs, is the pregenital circuit may have endured in the CNS from the time of abdominal locomotory circuits of a pancrustacean aquatic insect ancestor (Regier et al. 2010; Engel 2015; Schwentner et al. 2016; Serano et al. 2016). In this regard, the potential of the pregenital insect abdomen to develop new non-sexual appendages that are motile has been demonstrated (Hoch et al. 2014), and obviously the abdominal swimmerets of crustaceans are motile pregenital appendages (Mulloney and Smarandache-Wallmann 2012). Thus, descending inhibition could also be a strategy for suppressing abdominal circuits for behaviors that are no longer expressed as a result of evolution.

In larval and adult grasshoppers and locusts, the pregenital abdomen does produce at least one more CPG-based rhythmic behavior: ventilation of the tracheolar respiratory system (Hustert 1974, 1975; Bustami and Hustert 2000; Groenewald et al. 2012; Harrison et al. 2013). There has been an assumption that, while the primary CPG for ventilation resides in the metathoracic ganglion, secondary oscillators mediating ventilation are found in each abdominal ganglion (Miller 1960; Burrows 1996). The movements of the isolated abdomen, notably weaker than normal respiration, have been regarded as a second form of ventilation. Tracing back the references for independent abdominal ventilation-generation leads to the early studies by Baudelot (1864) on aquatic dragonfly larvae, which is the historical and perpetuated citation. More recent research on dragonfly larvae has determined that the rectal respiration rhythm is directed from a primary oscillator located in the terminal abdominal ganglion (Komatsu 1982). Although other investigators have assumed that the pregenital behavior corresponds to a type of ventilation in which telescoping movements of the abdomen supplement the dorso-ventral compressions, or that the contractions of longitudinal muscles act to prevent lengthening of the abdomen when the interior pressure builds in ventilation, there are two reasons why other explanations are more likely. One, a slow metachronal pattern of longitudinal contractions would not coordinate well with the rapid simultaneous dorso-ventral contractions of all abdominal segments. The telescoping movements, because they are metachronal rather than synchronous would provide little change in volume for the abdomen. Two, basal tonus of the longitudinal muscles (Hoyle 1983) would resist such extension anyway. While it is still possible that the oviposition-like motor pattern in pregenital segments represents some alternative form of auxiliary, supplemental or weak ventilation, such a case would require both oviposition and this form of ventilation to be based on one and the same abdominal oscillator or CPG, an otherwise unknown occurrence.

Observations of inter- and intra-segmental coordination of ventilation, molting and locomotion provide little in the way of resolution of the relation of pregenital and oviposition activity to other abdominal behaviors. Without further investigation, a cautious interpretation of the data is that the induced pregenital motor pattern is of undetermined behavioral relevance in extant animals. The surprising finding of oviposition-like CPGs in the pregenital ganglia of male and female grasshoppers is significant in that, regardless of their present role in behavior, in the genital segments of females they formed the foundation by which evolution shaped one of the most remarkable and specialized of insect behaviors.

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