Chapter 2
Early Development and Diversity of *Gryllus* Appendages

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Abstract Among insects, orthopterans such as *Gryllus bimaculatus* display an extraordinary diversity regarding the arrangement and morphology of their appendages. In the head region, previous studies have shown that despite the superficial similarities in the morphology of mandibulate mouthparts between holometabolous and hemimetabolous species, the development of these appendages may be regulated in different ways. At present, a comprehensive analysis in any hemimetabolous mandibulate species is lacking; therefore studying the mouthparts in *Gryllus* will significantly improve the current understanding of the evolution of mouthparts in insects. Orthopteran wings are also quite distinct, featuring the hardened, leathery protective forewings (FWs) and the membranous flying hind wings (HWs). Furthermore, the FWs in *Gryllus* are characterized by a complex vein-intervein arrangement, similar to the ancestral hardened wings observed in fossils, providing a unique opportunity to understand the evolution of wing sclerotization in basal insects. Finally, orthopterans feature one of the best-known examples of appendage modification in insects – the presence of the greatly enlarged jumping hind leg. Studies of gene expression and functional analyses suggest that this enlargement is controlled by the Hox gene *Ultrabithorax* (*Ubx*), which acts as a "trigger" for differential leg growth. Furthermore, rather than acting on all genes in the leg development network, *Ubx* seems to selectively upregulate growth factors such as *decapentaplegic* (*dpp*) and *Lowfat* in *Gryllus*. Hence, cricket hind leg can serve as an exceptional model for combined studies of both tissue growth and segmental patterning during embryonic leg development. Overall, this review formulates a general framework that can be used for future studies on the development and diversification of insect appendages.

Keywords *Gryllus bimaculatus* • Embryogenesis • Mandibulate mouthparts • T1–T3 legs • *Decapentaplegic* • *Wingless* • *Extradenticle* • *Dachshund* • *Distal-less* • *Ultrabithorax*

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2.1 Introduction

The appendages of the two-spotted field cricket, *Gryllus bimaculatus*, display extraordinary morphological diversity in the head and thoracic segments. Despite such individual segment variation, the morphology of mouthparts, wings, and legs in *Gryllus* conforms to a typical orthopteran body plan. In the head region, there are three segments that bear gnathal appendages: mandibles, maxillae, and labia. Crickets have mandibulate mouthparts that are used for chewing and characterized by stubby mandibles, which serve as grinding plates (Fig. 2.1a). The maxillary and labial appendages are similar and have branched morphology, with the latter also being fused into a single structure. Among the three thoracic segments, the prothorax (T1) is wingless, while the meso- (T2) and metathorax (T3) each carry a pair of wings. The forewing (FW) and hind wing (HW) exhibit distinct differences in shape, size, texture, and pigmentation (Fig. 2.1b). The FW is thickened and hardened, mainly black with the main function in protecting the HW. In contrast, the HW is transparent and membranous with a primary function in flight. In addition to wings, each of the three thoracic segments also bears a pair of legs. Whereas two pairs of four legs are generally rather similar, the hind (T3) legs are greatly enlarged and modified for jumping and represent the most distinctive orthopteran feature (Fig. 2.1c).

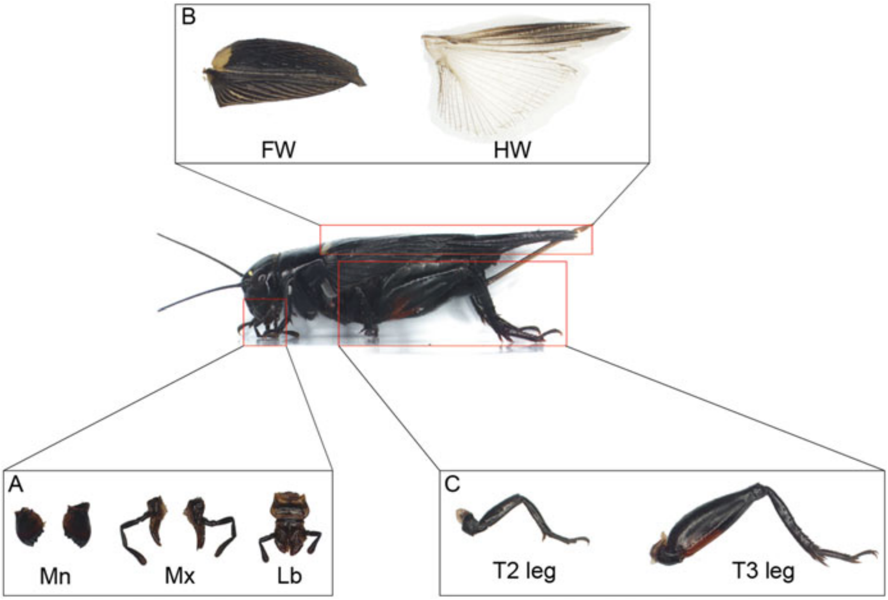

Fig. 2.1 The morphological diversity of *Gryllus* appendages. (**a**) The three gnathal appendages, mandible, maxillae, and labium, establish distinct morphologies. (**b**) The forewing and hind wing of *Gryllus* establish distinct shape and coloration. (**c**) The T2 and T3 legs show significant difference in size. Abbreviations: *Mn* mandible, *Mx* maxilla, *Lb* labium, *FW* forewing, *HW* hind wing

At present, the studies of the molecular mechanism that generate such distinct morphologies of appendages in *Gryllus* had almost exclusively focused on the development of the legs. The data on mouthparts is limited to a single expression study (Zhang et al. 2005), although more information is available in a related house cricket species, *Acheta domesticus* (Rogers et al. 1997, 2002). The development of wings in orthoptera is yet to be analyzed. At the same time, studies in other, mainly holometabolous model species have provided classical insights into the development of these appendages (de Celis et al. 1996; Ng et al. 1996; Kim et al. 1996; Neumann and Cohen 1998). Here we review the available information and discuss whether and to what degree the current developmental models can be applied to *Gryllus*. We also provide a framework for future studies in this and other orthopteran species that can be used to improve the current understanding of the development and differentiation of the head and thoracic appendages in hemimetabolous insects.

2.2 *Gryllus* Mouthparts

Gryllus mouthparts represent the ancestral form of gnathal appendages that are also found in other basal insect lineages such as cockroaches or primitively wingless silverfish. The more derived groups featured a trend toward further specialization, leading to the development of the haustellate (an adaptation to piercing and sucking; Hemiptera) or sponging (Diptera) mouthparts (Snodgrass 1993). Despite their distinct morphological differences, though, the identities of gnathal segments are controlled by the same set of three Hox genes in all insects studied so far (Hughes and Kaufman 2000; Rogers et al. 2002; Martinez-Arias et al. 1987; Merrill et al. 1987; Shippy et al. 2000, 2006; DeCamillis et al. 2001; Curtis et al. 2001; DeCamillis and ffrench-Constant 2003; Brown et al. 2000). Thus, it is now generally accepted that the evolution of mouthparts was governed by the changes in the expression (Rogers et al. 1997, 2002) and/or functions of *Deformed* (*Dfd*), *proboscipedia* (*pb*), and *Sex combs reduced* (*Scr*).

Previous studies of a number of mandibulate species, including the house cricket, *Acheta domesticus*, have shown that the expression patterns of these genes are conserved (Passalacqua et al. 2010; Rogers et al. 1997, 2002; Hrycaj 2010; Curtis et al. 2001; DeCamillis and ffrench-Constant 2003; DeCamillis et al. 2001; Shippy et al. 2000, 2006; Brown et al. 2000). First, *Dfd* is localized throughout the entire mandibular and maxillary segments and their appendages. Second, the expression of *pb* is more posterior and can be observed in the outer branches of the developing maxillary and labial appendages. Note that *pb* is never observed in the proximal portion of either appendage. Third, the *Scr* is primarily expressed in the labial segment. Of these three genes, only *Scr* pattern has been determined in *Gryllus*, and its localization in the labial segment follows the consensus observed in other mandibulate species (Zhang et al. 2005). Hence, it is likely that the expression patterns of *Dfd* and *pb* in *Gryllus* may also follow a mandibulate consensus pattern.

At present, the main functional insight into the genetic mechanisms that control the development of mandibulate mouthparts was generated through the studies of *Tribolium castaneum*, a holometabolous species (Curtis et al. 2001; DeCamillis and ffrench-Constant 2003; DeCamillis et al. 2001; Shippy et al. 2000, 2006; Brown et al. 2000) . Among hemimetabolous groups, the only functional data available is from *Oncopeltus fasciatus*, a hemipteran that has haustellate mouthparts (Hughes and Kaufman 2000). Hence, the potential new insight from a species such as *Gryllus* would be critical for an in depth understanding of evolutionary transition from mandibulate to haustellate insects. In addition, the presence of such information would allow for a direct comparison of genetic mechanisms that control the morphology of mandibular appendages in holo- (*Tribolium*) and hemimetabolous insects (*Gryllus*). In the former, the depletion of a head Hox gene generally causes a distinct identity transformation of the affected gnathal appendage(s). Specifically, *Dfd* RNAi transforms mandibles to legs, without any change in the identity of maxillae (Brown et al. 2000). Similarly, the loss of function of *pb* changes maxillary and labial palps into legs (DeCamillis and ffrench-Constant 2003; DeCamillis et al. 2001; Shippy et al. 2000), while loss of *Scr* results in the complete transformation of labium to antennae (Curtis et al. 2001; DeCamillis et al. 2001; Shippy et al. 2006). It is intriguing that in *Periplaneta americana* (cockroach), which is also a mandibulate insect, the labial appendage displays a mixture of leg and antennae morphology in *Scr* RNAi adults (Hrycaj et al. 2010). This finding suggests that insights from *Tribolium* cannot be directly applied and generalized to other mandibulate lineages. Since *Periplaneta* is also distantly related to orthopterans – it is likely that situation in *Gryllus* would be more similar to it than to *Tribolium*. For example, the loss of function of *Dfd* should result in antenna-like mandibles and maxillae with mixed leg/antenna morphology. The similar mixed identity should also be observed in maxillae in the absences of *pb* or in the labium in the absence of the *Scr*. In contrast, the double depletions of *Dfd*/*pb* and *Scr*/*pb* should transform maxillae and labium into antennae, respectively. Future studies confirming these predictions would provide a general framework for detailed understanding of the development of mouthparts in mandibulates and provide a critical insight in the evolution and diversification of gnathal appendages in insects in general.

2.3 Wing Morphology

As illustrated in Fig. 2.1b, the two pairs of wings in *Gryllus* exhibit very different morphologies. At present, the molecular mechanisms underlying such differences have not been studied in this species. However, the now classical studies in *Drosophila*, *Tribolium*, *and Precis* have shown that the Hox gene *Ultrabithorax* (*Ubx*) controls the identity of the HW (Weatherbee et al. 1998, 1999; Tomoyasu et al. 2005). This is further corroborated by the recent analysis of *Ubx* in *Acheta domesticus* (Turchyn 2010), in which *Ubx* RNAi transforms HW into FW in adults.

These findings support the notion that *Ubx* controls the hind wing identity by altering the forewing program in a species-specific manner. Hence, divergence of fore- and hind wings can now be understood and examined by analyzing the downstream genes that are up- or downregulated by *Ubx* in each lineage.

In Coleoptera (beetles), the FWs are modified into firm wing cases (elytra) that protect the hind wings underneath and as such can be used to gain an insight into the genetic mechanisms governing the "hardening" wing program. Recent studies in *Tribolium* show that *apterous* (*ap*) and *achete-scute homolog* (*ASH*) are two essential factors creating exoskeleton in elytra (Tomoyasu et al. 2005, 2009). Specifically, the depletion of *ap* can cause loss of exoskeleton of the intervein regions of the elytra, whereas *ASH* RNAi leads to removal of exoskeleton patches that surround the bristles. Based upon these observations, it has been proposed that the combined input from *ap* (functioning as the intervein selector) and *ASH* (functioning as the bristle selector) controls the sclerotization of subsequent elytron regions. Intriguingly, even when both *ap* and *ASH* are knocked down in *Tribolium*, the veins remain sclerotized (Tomoyasu et al. 2009). This suggested that there is another factor that is involved in the hardening of the veins (a putative vein selector). At present, it is not known if, and to what degree, such mechanisms can be generalized to other insects with hardened FW. In *Gryllus*, the vein-intervein arrangement is quite different from the one present in beetles. While the veins in *Tribolium* run parallel along the elytra forming the longitudinal intervein regions, the veins in *Gryllus* are arranged in a complex parallel and perpendicular pattern that divides the FW into checkered intervein territories. These perpendicular crossveins that generate such a meshwork in *Gryllus* do not exist in the *Tribolium* elytra. Hence, the hardening of FW in crickets may have a different genetic underpinning when compared to beetles, further highlighting the significance of determining the molecular basis of wing diversification in *Gryllus*.

Among the general public, crickets are perhaps best known for their chirping (stridulation), which is an integral part of their mating behavior. The cricket song is produced by special structures located in the male FW (Huber et al. 1989; Montealegre et al. 2011): the plectrum (scraper) of the left FW, the stridulatory file (teeth) of the right FW, and a resonator (harp and/or mirror) located on both FWs (Fig. 2.2). During stridulation, the scraper sweeps along the row of teeth to produce the sound, while the harp and mirror serve as the acoustic tuner. In addition to their location on the FW in crickets and katydids, these structures can occasionally be found in other body regions as well. For example, the scraper in grasshoppers is located on the hind legs, whereas the FW contains the file (Snodgrass 1930). Also in rare instances, as observed in sandgropers (*Cylindracheta psammophila*), the scraper and the file are located in the mouthparts (Rentz 1991). Future studies of genetic regulation of chirping in *Gryllus* would provide a greatly needed complement to studies of mating behavior in this species. Consequently, by utilizing the power of functional testing and genome engineering, the crickets have the potential to become one of the premier animal models for studying behavior at the genetic level. More details on chirping and behavior of *Gryllus* are discussed in later chapters.

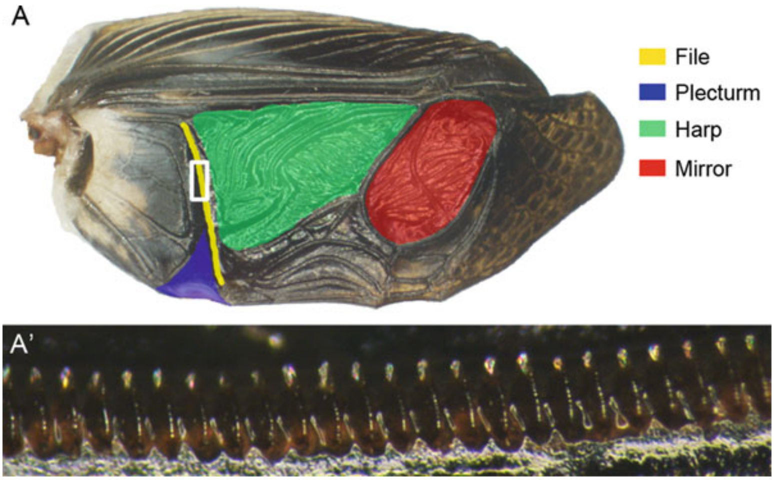

Fig. 2.2 The stridulatory organs on the forewing of male *Gryllus*. (**a**) The file (*yellow*), plectrum (*blue*), harp (*green*), and mirror (*red*) on the forewing. (**a'**) The magnified view of the file (*white rectangular* in (**a**))

2.4 Leg Morphology

In terms of its overall morphology, the *Gryllus*' most distinctive feature is the presence of the greatly enlarged, "jumping" hind legs (Fig. 2.1c). These appendages are based on the common hexapod leg design and represent one of the most recognizable examples of allometric growth in insects (Mahfooz et al. 2007; Turchyn 2010). All three pairs of legs in *Gryllus* share the same modular organization along the proximal/distal axis and are composed of six segments: coxa, trochanter, femur, tibia, tarsus, and claws. The classical functional studies in *Drosophila* (Struhl 1982), as well as more recent results from a variety of species (Chesebro et al. 2009; Hrycaj et al. 2010; Mahfooz et al. 2007; Khila et al. 2009) have established that the "default morphology" of all ventral appendages is the metathoracic (T2) leg. In orthopterans (crickets, katydids, and grasshoppers), the jumping hind legs are generated by the differential growth of the femoral and tibial segments. Here, we first discuss the proximal-distal (P/D) axis formation during early leg development, which establishes the proper positioning of leg segments. Then we focus on the enlargement of the T3 leg, which takes place during mid-late developmental stages.

2.4.1 Leg Patterning

Nearly all of the present understanding of insect leg patterning was inferred from classic experiments of *Drosophila* leg discs (reviewed in Morata 2001), which showed that the elaboration of the leg proximal/distal axis is governed by the activities of *Distal-less* (*Dll*), *dachshund* (*dac*), and *homothorax* (*hth*)/*extradenticle* (*exd*). *Dll* is expressed in the center of the leg disc, where it specifies the distal leg segments; *dac* is expressed in the middle disc region, forming the intermediate leg segments; and *hth*/*exd* are expressed on the disc periphery regulating the formation of the proximal most leg segments. The mechanism driving these patterns was described as a "gradient model" (Fig. 2.3) and was originally proposed by Lecuit and Cohen (1997). The essence of this model is the formation of the central-peripheral gradient of two key morphogens: Wingless (Wg) and Decapentaplegic

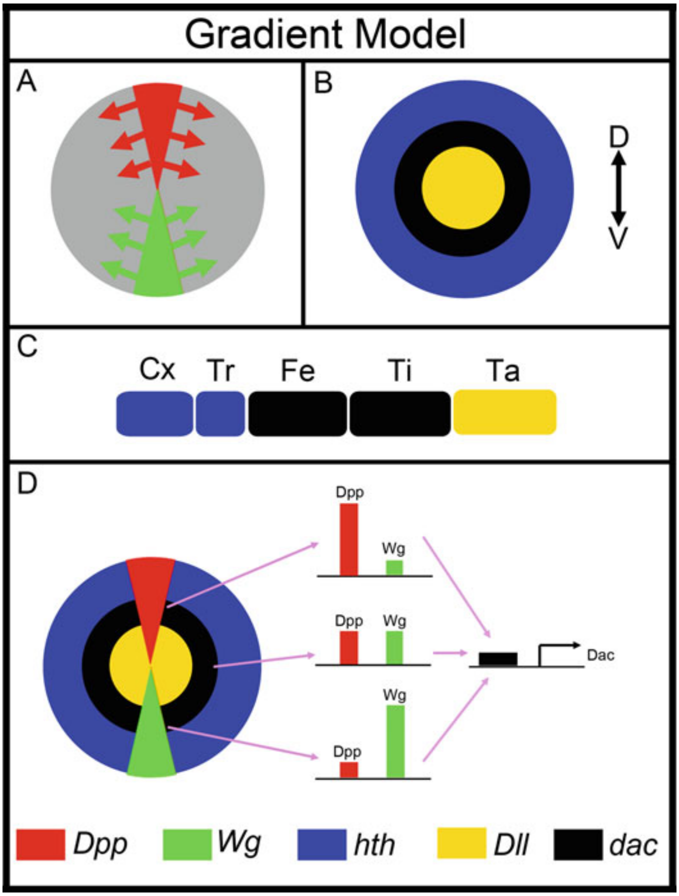

Fig. 2.3 The gradient model of *Drosophila* leg P/D axis formation, redrawn from Morata (2001). (**a**) The formation of *Wg* and *Dpp* gradient in *Drosophila* leg disc. The *arrows* illustrate how *Dpp* and *Wg* signals diffuse from their original (early) expression domains. (**b**) The gradient of *Wg* and *Dpp* concentration causes the activation of *Dll* in the center, *dac* in the middle, and *hth*/*exd* in the periphery of the leg disc, respectively. (**c**) The different subdomains in an adult leg, as illustrated by different segments. Generally, the coxa and trochanter are determined by *hth*/*exd*, femur and tibia by *dac*, and tarsal segments by *Dll*, respectively. (**d**) The main drawback of the gradient model, as illustrated by the difficulty in explaining *dac* expression pattern (Redrawn from Estella et al. 2012). The cis-regulatory module of *dac* must interpret very different ratios of Dpp:Wg signaling depending on the position in the leg disc. *Cx* coxa, *Tr* trochanter, *Fe* femur, *Ti* tibia, *Ta* tarsus

(Dpp). In the leg disc, these molecules are initially expressed as a dorsal (*dpp*) or ventral (*wg*) stripe, respectively. While the highest levels of Wg and Dpp are in the center, they begin to diffuse from their central location causing a drop in their expression levels in regions closer to the periphery of the disc (Fig. 2.3a). These differences in concentrations of Wg and Dpp, in turn, trigger the expression of *Dll* and *dac*, which display dose-dependent responses to the two morphogens (Fig. 2.3b). In the center of the leg disc (featuring the highest concentrations of Wg and Dpp), *Dll* is activated while *dac* is repressed. In the middle region (exhibiting the intermediate levels of Wg and Dpp), it is *dac* that is turned on while *Dll* is no longer expressed. In the periphery of the leg disc (characterized by the low levels of both morphogens), neither *dac* nor *Dll* is activated (Morata 2001; Lecuit and Cohen 1997). Instead, *hth* and *exd* are expressed in this region. Following the formation of such *Dll*-*dac*-*hth*/*exd* patterning, the leg segmentation becomes noticeable (Fig. 2.3c). While the gradient model has been broadly accepted over the last two decades to represent the general mode of leg development in insects, some of its aspects remained difficult to envision at the molecular level. As shown in Fig. 2.3d, in order to form a concentric *dac* expression domain in the middle region of the leg disc, the cis-regulatory module of *dac* must respond to distinct input levels of Wg and Dpp signals (Estella et al. 2012). This, however, is inconsistent with the key assumption that *dac* is activated by intermediate level of both Wg and Dpp. Therefore, the establishment of *Dll*-*dac*-*hth*/*exd* regulatory cascade in *Drosophila* leg imaginal discs cannot be solely explained by the central-peripheral gradient of Wg and Dpp. In contrast to the holometabolous mode, the appendages in hemimetabolous insects originate as limb buds that gradually extend in the distal direction during embryogenesis. In species such as *Gryllus*, *dpp* is initially localized only in the distal tip, whereas *wg* is expressed along the entire ventral margin of the limb bud (Niwa et al. 2000). Under such circumstance, only the Dpp signal would be capable of forming a proximal-distal gradient, since the level of Wg signal remains constant along the P/D axis. And yet, the conserved proximal-middle-distal expression of *hth*, *dac*, and *Dll* is still established, respectively. These observations in *Gryllus* suggest that the gradient model, at least in its strict sense, cannot fully account for the leg patterning in hemimetabolous species either.

To account for the observed inconsistencies of the gradient model, a new explanation was proposed recently (Estella et al. 2012). According to the "cascade model" (Fig. 2.4), Wg and Dpp are only required to turn on *Dll* and *epidermal growth factor receptor* (*EGFR*) in the center of the leg disc at the initialization of P/D axis. Consequently, *Dll* activates *dac* expression in the middle domain, whereas the repression of *dac* in the center region is maintained by the activity of EGFR. Compared to the gradient model, the cascade model is also more applicable to the observed situation of P/D axis patterning in *Gryllus*. At an early embryonic stage, both *wg* and *dpp* are expressed in the distal tip of the limb bud (Fig. 2.4, bottom), allowing them to act together and activate *Dll* to initialize the P/D axis (Inoue et al. 2002; Niwa et al. 2000). As the limb bud starts to elongate, neither of the morphogens are required for the maintenance of P/D axis. Instead, *Dll* activates *dac* expression in the intermediate leg region. At the same time, the repression of

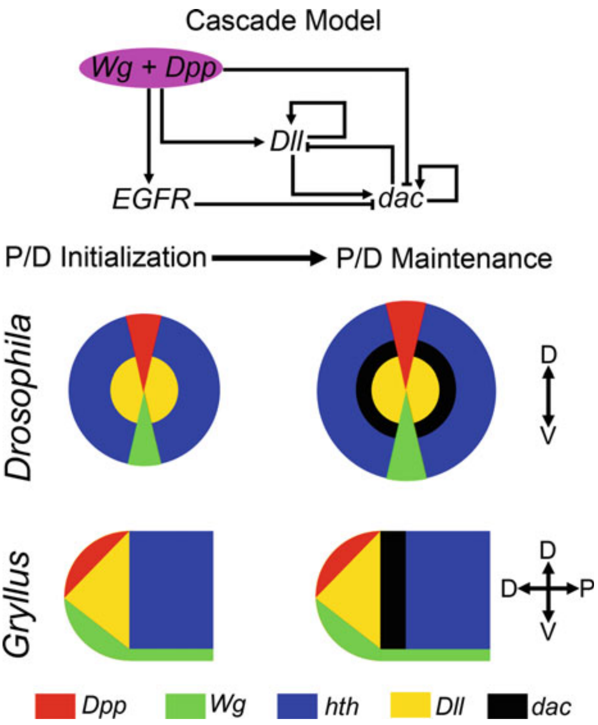

Fig. 2.4 The cascade model of P/D axis formation of an insect leg, redrawn from Estella et al. (2012). This model postulates the presence of two steps: initial phase (*left*) and maintenance phase (*right*). During the initial phase, the coexistence of *Wg* and *Dpp* in the center of *Drosophila* wing disc and distal tip of limb buds in *Gryllus* initiates P/D axis formation by activating *Dll* and *EGFR*. Later in the second phase, *Dll* activates *dac* in the middle concentric ring of *Drosophila* leg disc and middle region of *Gryllus* limb bud, after which both genes maintain their expression in a Wg+Dpp-independent manner. The expression of *hth*, in the periphery region of *Drosophila* leg disc and *Gryllus* limb bud, does not require Wg or Dpp. Note that the absence of *dac* in the distal domain is caused by joint Wg and Dpp repression during the initialization, while its expression at later stages of leg development is maintained by EGFR

dac in the distal region of *Gryllus* legs may be retained by the activity of EGFR, as recently reported by Nakamura et al. (2008b).

2.4.2 T3 Leg Allometric Growth

The key feature of insect T3 leg evolution is their lineage-specific differential enlargement. In basal insect lineages, such as thysanurans (firebrats) or archaeognathans (bristletails), all three pairs of legs are very similar in terms of their size and morphology (Mahfooz et al. 2004, 2007). Then, during the radiation of winged species, there was a trend toward lineage-specific enlargement of hind legs. This trend generated the situation that exists today, with differences between lineages encompassing both the involvement of different leg segments as well as varying degrees of enlargement of those segments. Orthopterans feature the largest differential growth of T3 legs, which can be almost twice as large as T1 or T2 legs. This, in turn, makes species such as *Gryllus* excellent models for elucidating the mechanisms of allometric leg growth.

From the conceptual standpoint, differential growth should be associated and coordinated with both leg patterning and joint formation. This is because a particular leg segment territory first has to be defined (i.e., separated by joints from other segments), before it can reach its final size. However, these processes have been traditionally studied separately, and presently very little is known about how they may be coordinated at the molecular level. The observation that *dpp* is differentially expressed in *Gryllus* hind legs at later embryonic stages provides a starting point for a more synergistic insight into the T3 leg enlargement (Niwa et al. 2000). As postulated by the cascade model (Fig. 2.4; (Estella et al. 2012)), the *dpp* plays an essential and conserved role during the initialization of the proximal-distal (P/D) axis in insects. During the mid-developmental stages, though, its pattern in *Drosophila* is transformed into a set of four complete circumferential rings in the tarsus while other leg segments exhibit only a patchy dorsal expression (Fig. 2.5). Recent studies have revealed that the circumferential expression domains are essential to create sharp boundaries of Dpp between leg segments, which induce a Jun N-terminal kinase (JNK)-reaper-dependent apoptosis required for the development of the leg joints (Manjon et al. 2007). Furthermore, this was also proposed to be the "ancestral mechanism" for joint formation in the distal leg regions. As illustrated in Fig. 2.5, while circumferential *dpp* expression is restricted to only tarsal segments in *Drosophila*, it expands to encompass both the femur and tibia in *Gryllus*. Similar observations were reported in another orthopteran, the grasshopper *Schistocerca americana* (Jockusch et al. 2000), suggesting that *dpp* may be involved in the formation of the femur-tibia and tibia-tarsus joints in more basal insect lineages.

The most significant divergence in *dpp* expression patterns in *Gryllus* is observed during later leg differentiation, when divisions between segments become more apparent (Niwa et al. 2000). While the previously complete circumferential rings turn into separate ventral and dorsal patches in T1 and T2 legs, this is not the case in hind legs where the rings are retained and become much wider (Fig. 2.5). As pointed by Niwa et al. 2000, these changes in expression also coincide with the increase in size of the hind legs. In light of the previously documented role of *dpp* signaling in wing tissue growth in flies (Hamaratoglu et al. 2011; Schwank and Basler 2010), it is tempting to postulate the similar causal relationship between the differential expression of *dpp* and differential growth of T3 legs in *Gryllus*. Similar results were observed in grasshoppers (Jockusch et al. 2000), but not in *Drosophila or Tribolium* (Manjon et al. 2007; Niwa et al. 2000), suggesting that this role of *dpp* may be unique to orthopterans.

The previous comparative analyses in several holo- and hemimetabolous insects have shown a tight association between the Hox gene *Ultrabithorax* (*Ubx*) and differential growth of hind legs (Mahfooz et al. 2004). In each instance, the

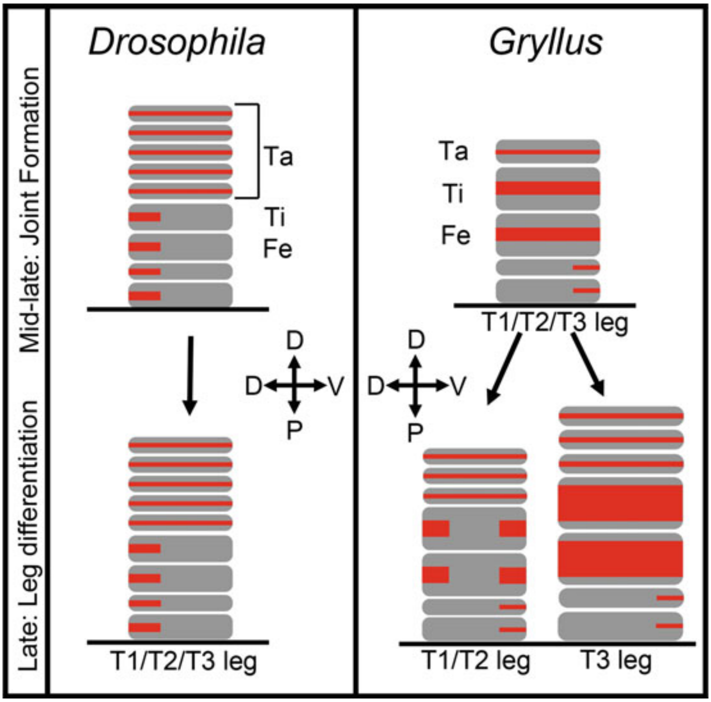

Fig. 2.5 The divergent expression patterns of *dpp* (*red*) between *Drosophila* and *Gryllus* during mid-late leg development, drawn accordingly to Manjon et al. (2007) and Niwa et al. (2000). During mid-late stage, the circumferential ringlike expression patterns of *dpp* are restricted in the tarsal segments in *Drosophila*, whereas they expand into the femur and tibia in *Gryllus*. During late stages, the expression pattern of *dpp* differentiates between T1/T2 leg and T3 leg in *Gryllus*, which is not found in *Drosophila*

expression of *Ubx* in particular leg segments is associated with the disproportionate enlargement of those segments. Furthermore, these enlarged segments display significant shortening and size reduction when *Ubx* is depleted (via RNAi) during embryogenesis (Khila et al. 2009; Mahfooz et al. 2004). These results confirm that *Ubx* can act as a “common trigger” for hind leg growth and diversification. In crickets, *Ubx* expression starts early during limb bud development and precedes *dpp* expression in T3 femur and tibia (Mahfooz et al. 2007; Zhang et al. 2005). In light of the functional studies that show that *Ubx* RNAi causes a reduction in size of these two segments in house crickets (Mahfooz et al. 2007), it is likely that similar mechanism may exist in *Gryllus* as well. Recent studies have suggested another growth factor, *Lowfat*, as a potential *Ubx* target due to its differential expression in T3 legs (Bando et al. 2011). At the same time, though, the majority of genes that were shown to be actually involved in leg growth and patterning (such as *EGFR*, *Fat*, *Dachsous*, and *Four-jointed*) exhibit common patterns in all legs (Nakamura et al. 2008a, b). These results indicate that *Ubx* may trigger the enlargement of T3 leg by selectively acting on a portion of growth regulators instead of upregulating the entire leg growth network. Thus, future studies should focus on determining whether *Ubx* can indeed activate the expression of *dpp* or *Lowfat* and if such activation is orthopteran specific or represents a more general way of generating differential growth of T3 legs.

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