Differential Developmental Programs in Two Closely Related Hawaiian Crickets

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ABSTRACT Hawaiian crickets in the genus *Laupala* Otte have emerged as a model system in the study of several evolutionary processes, such as the differentiation of signaling phenotypes, the evolution female preferences, and the phylogeographic patterns of speciation. However, very little is understood concerning the basic biology of species within this genus. Here, we document the postembryonic timing of developmental events in two closely related Hawaiian crickets in the genus *Laupala*. *Laupala kohalensis* Otte and *Laupala paranigra* Otte are closely related, exhibit widely divergent pulse rates of the male calling song, and are members of a rapid, recent, and extensive diversification of Hawaiian crickets. In this article, we used morphometric image analysis of developing individuals, from hatching through to adult maturation, to delineate instars in each species. We found that instar duration was consistent across both species, but the number of juvenile ecdysis events differed. *L. kohalensis* consistently exhibited eight instars. In contrast, *L. paranigra* exhibited a maximum of seven instars; moreover, half of the individuals experienced only six instars before maturation. A molecular mechanism linking the evolution of song pulse rate and developmental rate is discussed.

KEY WORDS *Laupala*, development, stridulation, ecdysis

OVER THE PAST DECADE, CRICKETS in the genus *Laupala* (Orthoptera: Gryllidae) have emerged as a model evolutionary system by which to study the processes of rapid phenotypic change, sexual selection, and speciation on islands. Since the formation of the Hawaiian archipelago 5 million years ago, 38 species within *Laupala* have diverged from a single common ancestor. Three *Laupala* species groups exist: the *kauai*, *pacifica*, and *cerasina* groups (Otte 1994). Two of these species groups, *pacifica* and *cerasina*, exhibit parallel phylogeographic patterns of diversification; older lineages in both species groups can be found on the older islands, younger lineages on the more recently formed islands (Shaw 2002). This pattern suggests that, as new islands were formed, they were colonized by interisland migrations. These rare interisland events were followed by more frequent within-island speciation. As a result, each *Laupala* species is a single island endemic (Otte 1994).

Sexual selection on male calling song is thought to have played a significant role in generating the species diversity observed within *Laupala* (Mendelson and Shaw 2002). Species within this genus are morphologically and ecologically very similar (Otte 1994), suggesting that ecological forces have not played a strong role in their diversification. Within sympatric communities, however, species can be easily distinguished based on male calling songs. A song consists of a simple train of pulses with no higher order structure. Males generally sing at the same carrier frequency. Species differ, however, in the rate at which pulses are produced (Shaw 1996a). Genetic studies confirm that both male pulse rate (Shaw 1996a) and female preference for a conspecific pulse rate (Shaw 2000) are genetically controlled.

A great deal is known about the evolutionary history of *Laupala* and the forces that have contributed to their rapid differentiation (Shaw and Danley 2003). However, very little is known about the ecology, life history, and developmental processes of *Laupala*. Further investigations in these areas are necessary to understand the proximate basis of the rapid differentiation of *Laupala* species.

The objective of this study was to examine the postembryonic development of two closely related species, *Laupala kohalensis* Otte and *Laupala paranigra* Otte, that produce dramatically different calling songs. This species pair has been the subject of several studies that document the evolutionary importance and genetic basis of male calling song (Shaw 1996a, Shaw and Parsons 2002, Shaw and Danley 2003). Although the recent evolutionary history shared by these species might lead us to expect broad similarities in postembryonic development, the divergence of songs characterizing these species could result from substantial changes in developmental program. The neural circuitry responsible for cricket stridulation is gradually constructed over the final one-third of the

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cricket postembryonic period (Bentley and Hoy 1970). A modification of male song may produce a concomitant change in postembryonic development or vice versa. Yet, very little developmental information is known for any *Laupala* species. Using morphometric measures of scleritic elements, we delineated the number and duration of stadia in *L. kohalensis* and *L. paranigra* and compared their developmental programs. A developmental analysis of these species may provide additional insight into the processes that have played a role in the rapid differentiation of species in this genus.

Materials and Methods

L. kohalensis and *L. paranigra* have diverged recently. Although not sympatric, they are both endemic to the island of Hawaii, suggesting that their differentiation has occurred within the past 500,000 yr. *L. kohalensis* is endemic to the Kohala mountains. The population used in this study was collected in the North Kohala District (elevation 1,800–1,900 ft; 155° 46′ W, 20° 10′ N). *L. paranigra*'s distribution is restricted to the southeastern portion of Hawaii, on the southeastern slopes of Mauna Kea, and eastern slopes of Mauna Loa. Individuals used in this study were collected in the South Hilo District (Kaiwiki) (elevation 2,100–2,300 ft; 155° 10′ W, 19° 46′ N). Initial phylogenetic studies based on mitochondrial DNA sequence variation had suggested that they were sister species (Shaw 1996b). Subsequent analysis of nuclear markers (anonymous noncoding sequence and AFLP) has indicated that although *L. kohalensis* and *L. paranigra* are not sister species, they are very closely related (Shaw 2002, Mendelson and Shaw 2005).

Both species occur in similar habitats and are nearly indistinguishable based on morphological measures (Otte 1994). They are easily distinguished, however, by the pulse rate of male song. Male *L. kohalensis* produce pulses at ≈3.7 pulses per second at 20°C. Male *L. paranigra* produce pulses at ≈0.8 pulses per second at 20°C. Because of their close phylogenetic relationship and the large difference in male pulse rates, these species have been the subject of a number of studies on the genetic basis of male calling song and female mating preferences (Shaw 1996a, 2000; Parsons and Shaw 2002; Shaw and Parsons 2002).

Stocks of *L. paranigra* and *L. kohalensis* were derived from naturally inseminated females collected in August 2002 and January 2003, respectively, from which sib stocks were established in the laboratory. Females of both species deposited fertilized eggs in a moist tissue (Kimwipes EX-L, Kimberly-Clark, Roswell, GA). Approximately twice per month the tissue was collected from each female's rearing container (a plastic specimen cup) and transferred to a separate cup. The cups containing fertilized eggs were checked daily for newly hatched nymphs. Newly hatched nymphs were housed individually in specimen cups. Stocks of both species and their resulting offspring were reared in the same environmentally controlled room (a photoperiod of 12:12 [L:D] h, ≈20°C) and were fed twice weekly with Purina Cricket Chow (Fluker's cricket ranch, Baton Rouge, LA). Moisture for all individuals was provided by a damp tissue in each cup.

Approximately once every other day beginning on the day an individual had hatched, each nymph was photographed using a JVC color video camera (model TK-128OU, JVC, Boston, MA) mounted to a Leica dissecting scope (model MZ8, Leica, Bannockburn, IL). Digital images of the nymphs were captured using NIH Image 1.62 software (http://rsb.info.nih.gov/nih-image) running on an Apple Power Macintosh computer (model 7500, Apple, Palo Alto, CA). Pictures were taken until each individual either died or reached adult maturity, as indicated by complete wing development, generating ≈57 photographs over 143 d per individual (*L. kohalensis*) or 50 photographs over 127 d per individual (*L. paranigra*). For each picture, the magnification and number of days since emergence were noted. A ruler was included in each picture for scale. The development of eight *L. paranigra* individuals was followed. Of these eight, five survived to adulthood (three males, two females). Nine *L. kohalensis* were followed. Eight survived to adulthood (six males, two females).

Instars were identified based on morphometric measures of three scleritic elements that have shown stepwise increases across development in other species (Mbata 1992, Suzuki and Nishimura 1997). Head width was measured as the widest part of the head perpendicular to the long axis of the body. Pronotum width was measured as the widest part of the pronotum perpendicular to the long axis of the body. Ovipositor length was measured in females (Fig. 1). The morphometric identification of instars was necessary because individuals consume their exuviae immediately after a molt.

Each individual was measured for each character at least once from each picture. An initial analysis of the data was performed on five *L. paranigra* individuals and five *L. kohalensis* individuals. For this preliminary analysis, head width, pronotum width, and ovipositor length were measured in each picture of an individual a minimum of three separate times by at least two separate persons. To detect changes in morphometric measures across consecutive photographs, *t*-tests were preformed. This allowed the construction of preliminary nymphal stages for each species. Preliminary nymphal stages were erected when significant *t* values were calculated for each of the possible measurements.

For the final analysis, the entire data set within a species was pooled and analyzed. For those individuals that had been measured more than once, the average of the measurements was used for the analysis. Breaks between nymphal stadia were identified as large increases (typically 100 μm) in a character across consecutive pictures in each individual's developmental series. These breaks often corresponded to the nymphal stadia previously identified in the preliminary analysis. Measurements for a given character within a given developmental stadium were av-

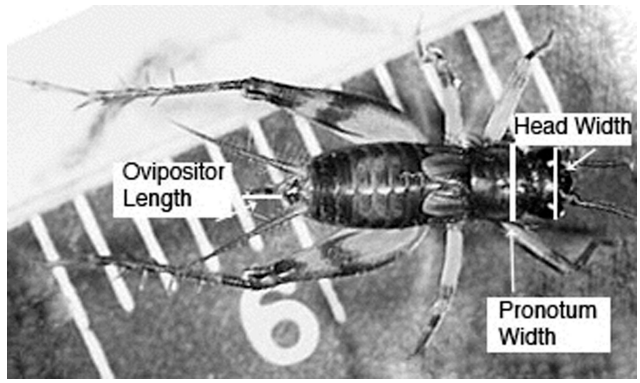

Fig. 1. Morphometric measurements. Three measures were made of each individual when possible: head width (HW), pronotum width (PW), and ovipositor length (OL).

eraged. This average was used in subsequent analysis as that individual's size at that nymphal stadium. Within a species, homologous nymphal stadia were easily aligned across individuals, such that a distribution of character measurements per nymphal stadium could be generated. *t*-tests across consecutive stadia tested the hypothesis that the size changes across the nymphal stadia were significant. A Bonferroni correction for multiple comparisons was made to establish statistically meaningful significance thresholds.

The duration of each stadium also was estimated. The number of days since hatching was recorded for each day an individual was measured. The duration of a given instar was then back-calculated by subtracting the first day that the instar began from the first day of the subsequent instar.

Results

For this study, measurements of males and females were pooled within species. Low sample sizes, particularly of females, necessitated this pooling. Preliminary analysis (not shown) of gender differences further justified the pooling of male and female data. Within *L. kohalensis*, males tended to have slightly wider heads at each stage, but the difference in width tended to be small (within 5–25 μm). These differences amounted to less than one standard deviation separating the sexes' head widths. A comparison of *L. kohalensis* pronotum widths across genders revealed no gender bias in size. Males were larger in five of the comparisons. Females were larger in four of the comparisons. These differences were again minor (5–33 μm) and accounted for less than a standard deviation. *L. paranigra* exhibited similar degrees of size differences between the sexes. The differences in head widths between male and female *L. paranigra* were minor (6–43 μm), and neither gender was uniformly larger. Females had larger heads in four of the instars. Males had larger heads in three of the instars. A comparison of *L. paranigra* pronotum widths yielded similar results; the differences in size ranged from 2 to 73 μm, and neither gender was uniformly larger. Females were larger in four of the instars; males were larger in three of the instars.

L. kohalensis. Eight juvenile instars were identified based on morphometric measurements in *L. kohalensis* (Figs. 2, 3, and 4). A significant increase in both head with and pronotum width was observed with each ecdysis event (Figs. 2 and 3). Ovipositor lengths also increased with each successive developmental stadium (Fig. 4); however, these increases in ovipositor length were not statistically significant. The lack of significance is likely the result of the low sample size of females in the study. Wing buds and, in females, ovipositors occurred at instar 6. The duration of each stadium was relatively consistent (Table 1). On average, the postembryonic development of *L. kohalensis* lasted for 143 d. Each stadium lasted ≈18 d.

L. paranigra. Seven instars were identified in *L. paranigra* (Figs. 2–4) based on morphometric measurements. Each instar displayed a significant increase in head width (Fig. 2), with the exception of the transition between instar 4 and 5. Likewise, pronotum widths and ovipositor lengths experienced a significant increase with each ecdysis (Figs. 3 and 4). Like *L. kohalensis*, wing buds and ovipositors occurred at instar 6. The average length of *L. paranigra* postembryonic development was 127 d (Table 1). The length of each instar was generally consistent across postembryonic development, with a mean of 19 d per instar.

Not all *L. paranigra* individuals experienced seven instars. Half of the individuals that survived past instar 6 were developmentally "precocious" in that they apparently skipped instar 5. Precocious individuals possessed significantly larger pronota than individuals with protracted development before instar 5 (1.021 versus 0.949 mm, $t = 4.69$, df = 4, $P = 0.005$), but not wider heads (1.098 versus 1.064 mm, $t = 1.27$, df = 4, $P = 0.27$). Precocious individuals were significantly smaller after instar 5 (head width: 1.279 versus 1.363 mm, $t = 4.80$, df = 4, $P = 0.004$; pronotum width: 1.225 versus 1.293 mm, $t = 4.03$, df = 4, $P = 0.01$). They developed wing buds significantly earlier than individuals with the extended development (on average, day 74 versus 92.3, $t = 3.05$, df = 4, $P = 0.02$) and

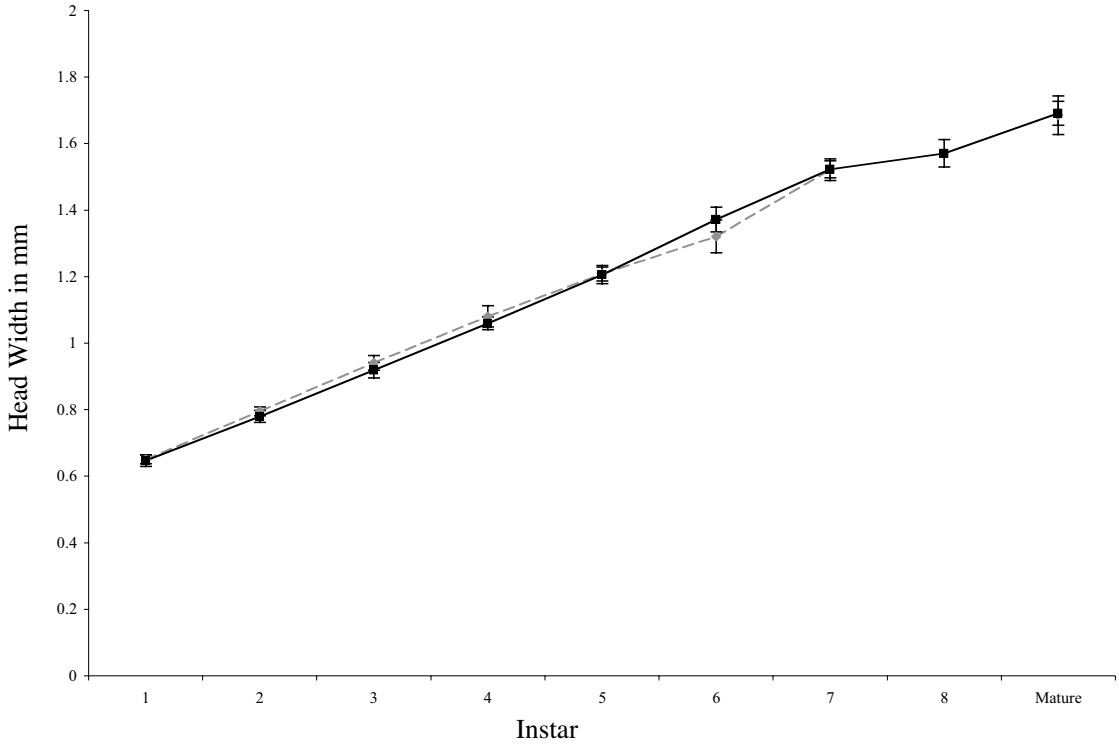

Fig. 2. Head widths across developmental time. The mean head width for a given instar in each species is presented. Error bars are equal to the standard deviation of head widths in a given stage. *L. kohalensis* head widths (black squares) significantly increased with each successive instar [instars 1 through maturity, *P* value, *t*, degrees freedom: (1–2) 1.38×10^{-9}, 30.67, 8; (2–3) 3.17×10^{-9}, 27.63, 8; (3–4) 3.87×10^{-8}, 20.12, 8; (4–5) 9.72×10^{-9}, 23.99, 8; (5–6) 8.68×10^{-8}, 18.16, 8; (6–7) 6.49×10^{-7}, 16.79, 7; (7–8) 3.00×10^{-4}, 6.65, 7; (8-mature) 1.00×10^{-4}, 7.53, 7]. Significance levels for *L. kohalensis*, after Bonferroni correction for eight comparisons, were 6.00×10^{-3} ($P = 0.05$) and 1.25×10^{-3} ($P = 0.01$). *L. paranigra* head widths (gray diamonds) increased with each successive instar. All increases were statistically significant except the increase from instar 4–5, which was nearly significant [instars 1 through maturity, *P* value, *t*, degrees freedom: (1–2) 1.03×10^{-8}, 30.58, 7; (2–3) 2.56×10^{-8}, 37.16, 6; (3–4) 7.64×10^{-7}, 20.98, 6; (4–5) 9.20×10^{-3}, 10.34, 2; (5–6) 1.00×10^{-3}, 31.70, 2; (6–7) 4.96×10^{-5}, 12.91, 5; (7–mature) 9.00×10^{-4}, 8.81, 4]. Significance levels for *L. paranigra*, after Bonferroni correction for seven comparisons, were 7.14×10^{-4} ($P = 0.05$) and 1.43×10^{-3} ($P = 0.01$).

achieved maturity earlier (day 116.5 versus 134, $t = 3.86$, df = 2, $P = 0.03$). The average length of an instar among precocious individuals did not differ from those with extended development (18.9 d [precocious] versus 19 d, $t = 0.044$, df = 36, $P = 0.96$). The ability to skip instar 5 does not seem to be sex-limited. Two of the individuals were female. The third was male.

Interspecific Comparisons. As one might expect, given the close phylogenetic relationship of these two species, the postembryonic development of *L. kohalensis* and *L. paranigra* share many features. Both exhibit discrete instars that are easily distinguishable based on morphometric measurements. These instars can be aligned between species (Table 2). The size of each character at each instar is equivalent across the species at all instars for both head widths and ovipositor length. The species have equivalently sized pronota during early development but diverge during later instars. *L. kohalensis* possess larger pronotum widths beginning with instar 5. Both species have extended postembryonic development that spans over 100 d. The average length of each stadium was similar in each species (*L. kohalensis* 18 d, *L. paranigra* 19 d).

Significant differences in the development of these two closely related species were observed. *L. kohalensis* consistently exhibited eight postembryonic stadia; *L. paranigra* exhibited as many as seven instars, but several individuals bypassed one instar (instar 5) and matured earlier. Consistent with the decreased number of instars in *L. paranigra* relative to *L. kohalensis*, *L. paranigra* developed significantly more rapidly (127 d) relative to *L. kohalensis* (143 d) ($t = 3.038$, df = 8, $P = 0.016$). The early maturing *L. paranigra* had a more abbreviated postembryonic period (116 d).

Discussion

L. kohalensis and *L. paranigra* differ tremendously in male calling song and female acoustic preference (Shaw 1996a, 2000) but are otherwise extremely similar. Their restricted distribution to the Big Island of

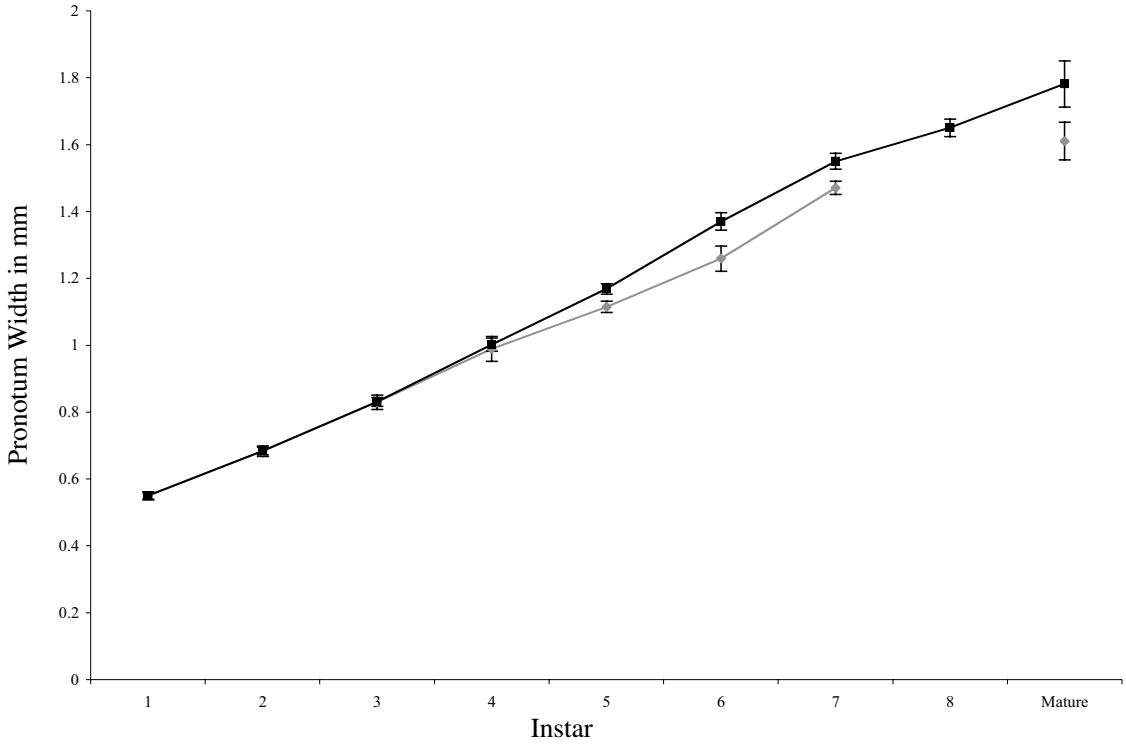

Fig. 3. Pronotum widths across developmental time. The mean pronotum width for a given instar in each species is presented. Error bars are equal to the standard deviation of pronotum widths in a given stage. *L. kohalensis* pronotum widths (black squares) were significantly larger with each successive instar (significance levels are identical to those described for Fig. 2) [instars 1 through maturity *P* value, *t*, degrees freedom: (1–2) 8.64×10^{-11}, 43.48, 8; (2–3) 5.36×10^{-9}, 25.86, 8; (3–4) 3.97×10^{-9}, 26.86, 8; (4–5) 1.30×10^{-8}, 23.11, 8; (5–6) 8.73×10^{-8}, 18.15, 8; (6–7) 2.87×10^{-7}, −18.91, 7; (7–8) 9.34×10^{-6}, 11.33, 7; (8–mature) 7.00×10^{-4}, 5.70, 7]. Pronotum widths also increased with each successive instar in *L. paranigra* (gray diamonds). All increases were statistically significant after multiple comparisons (significance thresholds as described in Fig. 2) [instars 1 through maturity, *P* value, *t*, degrees freedom: (1–2) 1.74×10^{-8}, 28.36, 7; (2–3) 8.53×10^{-8}, 30.32, 6; (3–4) 2.27×10^{-6}, 17.45, 6; (4–5) 5.0×10^{-4}, 43.85, 2; (5–6) 4.4×10^{-3}, 15.00, 2; (6–7) 6.21×10^{-6}, 19.71, 5; (7–mature) 1.7×10^{-3}, 7.45, 4].

Hawaii, their close evolutionary relationship, and their ability to hybridize in the laboratory indicates that they likely diverged from a common ancestor within the past 500,000 yr (Shaw 2002). Consequently, selection on the acoustic system is implicated in causing the differentiation between *L. paranigra* and *L. kohalensis*, possibly through the action of sexual selection. Although much is now known about their evolutionary history, many other elements of these species' basic biology have not yet been studied. Here, we examined the postembryonic developmental programs of *L. kohalensis* and *L. paranigra*, quantifying their similarities and differences.

Three morphological measurements (head width, pronotum width, and ovipositor length) were made of each individual at regular time periods during their postembryonic development. In both species, these measurements revealed discrete break points at regular time intervals. We interpret these break points as indications of ecdysal events, such that developmental stadia can be delineated by these discrete changes in size.

Owing to their close phylogenetic relationship, we expected these species' postembryonic development to be similar. In both species, an increase of both head and pronotum width occurred with ecdysis. With the exception of pronotum size after the sixth molt, the size distribution of both head and pronotum width was similar across both species. These measures should provide an unambiguous estimate of an individual's developmental stage in both species. The ovipositor length of the female proved less reliable as an indicator of developmental stage. However, this is likely due to the low sample size of females at this stage rather than the ovipositor being an intrinsically poor marker of a given instar. Both species exhibited fairly regular timing to their molts. In our study, a stadium typically lasted 18–19 d in each species under set environmental conditions. The time since hatching, however, does not provide an accurate estimate of an individual's developmental stage, because the number of instars may vary. Morphometric measures, however, seem to provide the more reliable estimate of developmental stage.

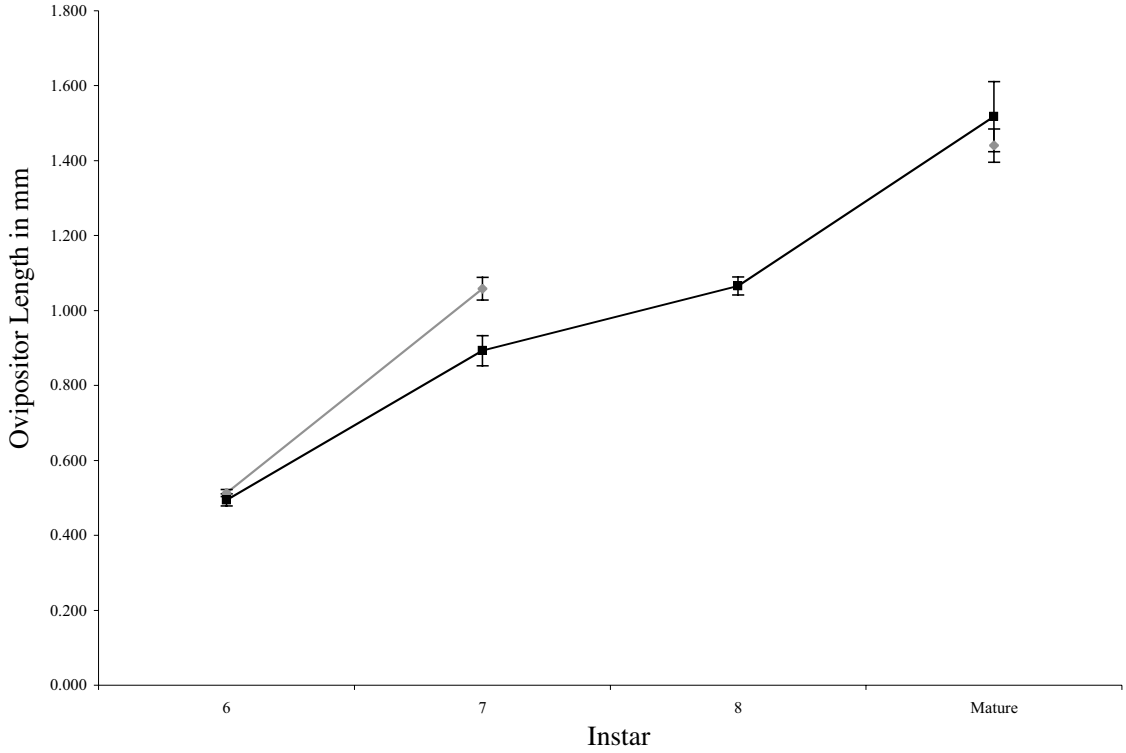

Fig. 4. Ovipositor lengths across developmental time. The mean ovipositor length for a given instar in each species is presented. Error bars are equal to the standard deviation of ovipositor lengths in a given stage. Ovipositor lengths increased with each stage in both species. Within *L. kohalensis* (black squares), however, none of the increases were statistically significant [instars 6 though maturity, *P* value, *t*, degrees freedom: (6–7) 0.09, 6.98, 1; (7–8) 0.06, 10.18, 1; (8–mature) 0.09, 6.46, 1)]. Significance thresholds, after correcting for three comparisons, were 0.016 ($P = 0.05$) and 0.003 ($P = 0.01$). Ovipositor lengths significantly increased in each successive instar within *L. paranigra* (gray diamonds) [instars 6 though maturity, *P* value, *t*, degrees freedom: (6–7) 0.025, 25.33, 1; (7–mature) 0.023, 27.35, 1]. Significance thresholds, after correcting for two comparisons, were 0.025 ($P = 0.05$) and 0.005 ($P = 0.01$).

Several logistical considerations contributed to the small sample size used in this study. *Laupala* generally have extended postembryonic periods (K.L.S., personal observation). This is true of the species in this study, both of which required >120 d after hatching to reach adulthood. The prolonged duration of their postembryonic development, the need to rear individuals separately, the necessity to photograph each individual at closely spaced time intervals, and the large number of photographs to be analyzed all contributed to this study's small sample size. The resulting lack of power precluded the likelihood of detecting statistical differentiation of certain morphological features through time (e.g., ovipositor length) and may have contributed to the small degree of differentiation observed in other comparisons (e.g., gender differences). The low sample size, however, did not prevent achieving the primary objective of the study. A clear,

Table 1. Duration of instars

Stage	*L. paranigra* - nonprecocious		*L. paranigra* - precocious		*L. kohalensis*	
	First day	Duration	First day	Duration	First day	Duration
1	1.00	19.00 (0.00)	1.00	21.00 (2.64)	1.00	20.00 (0.50)
2	20.19	18.67 (2.51)	22.00	15.33 (1.15)	21.00	16.00 (1.00)
3	38.54	15.33 (1.15)	37.33	19.00 (3.00)	37.00	19.89 (4.69)
4	54.36	18.00 (2.64)	56.33	17.67 (4.16)	56.89	17.78 (1.48)
5	72.00	20.33 (6.65)		0.00	74.67	18.11 (2.15)
6	92.33	16.00 (6.24)	74.00	19.00 (2.64)	92.78	22.13 (3.09)
7	106.42	25.67 (3.78)	93.00	23.00 (2.82)	115.13	16.25 (3.41)
8					131.38	12.00 (2.88)
Mature	132.25		116.50		143.38	

For each species, the average day that a given instar began is listed as well as its average duration (standard deviation). The length of each instar is pooled across genders within each species.

Table 2. Interspecific comparison of mean sizes of the three morphometric measures: head width, pronotum width and ovipositor length

Instar	*L. paranigra*	*L. kohalensis*	*P*	(*t*, df)
Head width				
1	0.65	0.65	0.64	(0.47, 15)
2	0.80	0.78	0.07	(1.93, 15)
3	0.94	0.92	0.07	(1.93, 14)
4	1.08	1.06	0.12	(1.64, 14)
5	1.21	1.21	0.92	(0.11, 10)
*6	1.32	1.37	0.04	(2.23, 13)
7	1.52	1.52	0.92	(0.10, 12)
8		1.57		
Mature	1.69	1.69	0.81	(0.23, 3)
Pronotum width				
1	0.55	0.55	0.98	(0.02, 15)
2	0.68	0.68	0.95	(0.06, 15)
3	0.83	0.83	0.88	(0.16, 14)
4	0.99	1.00	0.41	(0.85, 14)
5	**1.12**	**1.17**	**7.00×10^{-4}**	**(4.78, 10)**
***6**	**1.26**	**1.37**	**2.22×10^{-5}**	**(6.43, 13)**
7	**1.47**	**1.55**	**3.57×10^{-5}**	**(6.36, 12)**
8		1.65		
Mature	**1.61**	**1.78**	**9.00×10^{-4}**	**(4.48, 11)**
Ovipositor length				
*6	0.513	0.494	0.43	(0.98, 2)
7	1.058	0.893	0.08	(3.25, 2)
8		1.066		
Mature	1.441	1.518	0.53	(0.74, 2)

Significant differences, after Bonferroni correction, for eight comparisons (head width and pronotum width) or three comparisons (ovipositor length) between the species are given in bold.

statistically supported, delineation of instars in each species was accomplished through an analysis of morphological features.

The observed differences in developmental programs were unexpected. Crickets generally experience between five and 14 instars (Masaki and Walker 1987). The number of instars within a species can be plastic and is influenced by a number of environmental variables, including maternal investment in developmental resources (Holbrook and Schal 2004), availability and quality of food (Merkle 1977), photoperiod (Taniguchi and Tomioka 2003), rearing temperature (Merkle 1977), and social conditions (Holbrook and Schal 2004). However, all of these variables were held constant in this study. Within the species examined here, the maximum number of instars exhibited by any *L. kohalensis* individual was eight. *L. paranigra* exhibited fewer instars, with an observed maximum of seven. We observed variation in *L. paranigra* instar number, however, with half the individuals exhibiting six instars. Although we are unable to address the causes of developmental variation given the present design, genetic variation in developmental programs has been documented in other orthopterans. Simons et al. (1998) suggests that significant variation of postembryonic growth rates within populations of *Gryllus pennsylvanicus* Burmeister is genetically based. Similarly, the variation in instar number within *L. paranigra* may have resulted from segregating genetic variation in developmental program. *L. kohalensis* may lack genetic variation in the timing of developmental events, but a larger sample size is needed to test this hypothesis adequately. Nonetheless, both species would likely exhibit environmentally mediated plasticity in their postembryonic development that might be revealed under more variable rearing conditions.

The natural timing of developmental events in each species remains unknown. The rearing conditions used in this study were designed to mimic natural conditions but likely deviate in a small, but developmentally meaningful, way from natural conditions. For example, in the field it is unlikely that either species has ad libitum access to a high-protein food source similar to that provided in the laboratory. Nonetheless, the observed inter- and intraspecific differences in developmental timing are likely under genetic control. Although environmental conditions can produce developmental variation, the common laboratory conditions experienced by all of the individuals in this study suggest that the underlying genetic basis of developmental programs has diverged between these species. The evidence suggests that *L. kohalensis* possesses both a longer developmental period and a greater degree of canonization of its developmental program, whereas *L. paranigra* requires fewer maximum molts to reach maturity and exhibits greater plasticity in its development.

The evolution of stridulation rate and developmental timing, like that observed in *Laupala*, may be related through a molecular mechanism common to both processes. Both pulse rate and developmental rate rely on the specific timing of events. Recent molecular evidence suggests that such chronobiological events may be related. For example, postembryonic development in *Caenorhabditis elegans* (Maupas) is influenced by *lin*-42, a gene with high similarity to the *period* (*per*) locus that confers rhythmicity to male

song in *Drosophila* species (Jeon et al. 1999). *lin*-42 encodes a heterochronic protein that is believed to play a role in coordinating molting, particularly the final postembryonic molt. *per*, although well known for its role in circadian, locomotory, and eclosion rhythms (Konopka and Benzer 1971), also has been implicated in playing a role in species-specific male songs in *Drosophila* (for review, see Tauber and Eberl 2003). When *per* from *Drosophila simulans* Sturtevant was introduced into the genome of *Drosophila melanogaster* (Meigen) null *per* mutants, the *D. melanogaster* males produced songs with *D. simulans* pulse rates (Wheeler et al. 1991). *lin*-42 and *per* are similar in both DNA sequence and patterns of expression. *lin*-42 has the highest sequence similarity in the *C. elegans* genome to *per*, and, like *per*, *lin*-42 exhibits an oscillating pattern of expression. *lin*-42 cycles, however, are timed to ecdysis rather than circadian events (Jeon et al. 1999).

The pleiotopic relationship between pulse rate and development, if confirmed, could play an important role in shaping both developmental and song phenotypes. A correlated response to selection on male pulse rates, as a result of this pleiotropy, may account for the differentiation of developmental programs in these two closely related species. Sexual selection is known to be a powerful force in generating the rapid differentiation of signaling phenotypes (Streelman and Danley 2003) and may account for the rapid change of male pulse rates over a geologically brief time period (Shaw 2002). If pulse rate and developmental timing are related through the pleiotropic action of a gene common to both phenotypes, developmental rates may rapidly evolve in response to the differentiation of male pulse rates.

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