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Diurnal and developmental differences in gene expression between adult dispersing and flightless morphs of the wing polymorphic cricket, *Gryllus firmus*: Implications for life-history evolution

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ABSTRACT

The functional basis of life history adaptation is a key topic of research in life history evolution. Studies of wing-polymorphism in the cricket *Gryllus firmus* have played a prominent role in this field. However, prior in-depth investigations of morph specialization have primarily focused on a single hormone, juvenile hormone, and a single aspect of intermediary metabolism, the fatty-acid biosynthetic component of lipid metabolism. Moreover, the role of diurnal variation in life history adaptation in *G. firmus* has been understudied, as is the case for organisms in general. Here, we identify genes whose expression differs consistently between the morphs independent of time-of-day during early adulthood, as well as genes that exhibit a strong pattern of morph-specific diurnal expression. We find strong, consistent, morph-specific differences in the expression of genes involved in endocrine regulation, carbohydrate and lipid metabolism, and immunity – in particular, in the expression of an insulin-like-peptide precursor gene and genes involved in triglyceride production. We also find that the flight-capable morph exhibited a substantially greater number of genes exhibiting diurnal change in gene expression compared with the flightless morph, correlated with the greater circadian change in the hemolymph juvenile titer in the dispersing morph. In fact, diurnal differences in expression within the dispersing morph at different times of the day were significantly greater in magnitude than differences between dispersing and flightless morphs at the same time-of-day. These results provide important baseline information regarding the potential role of variable gene expression on life history specialization in morphs of *G. firmus*, and the first information on genetically-variable, diurnal change in gene expression, associated with a key life history polymorphism. These results also suggest the existence of prominent morph-specific circadian differences in gene expression in *G. firmus*, possibly caused by the morph-specific circadian rhythm in the juvenile hormone titer.

1. Introduction

For decades, the functional (physiological, biochemical, molecular) basis of life history adaptation has been a central topic of research on life history evolution (Stearns, 1992; Zera and Harshman, 2001; Harshman and Zera, 2007; Flatt and Heyland, 2011). However, despite considerable study, only the basic outlines are known for many key aspects of this topic, such as the mechanisms responsible for allocation trade-offs that contribute to life-history trade-offs, and the endocrine regulation of life history specialization (see above references). Moreover, other important aspects of life history adaptation, such as the role of diurnal (daily-rhythmic) expression of life-history traits and the genes that contribute to them, remain virtually unstudied (Zera et al., 2017).

Wing polymorphism in insects has figured prominently in functional studies of life history adaptation. The polymorphism, which consists of a flight-capable and one or more flightless morphs that trade-off aspects of flight (production of wings, flight muscles, flight fuels; flight per se) for reproductive output, is a phylogenetically widespread adaptation of central importance in insects (Harrison, 1980; Hardie and Lees, 1985; Roff, 1986; Zera and Denno, 1997; Guerra, 2011; Zera and Harshman, 2001, 2009, 2011; Zera and Brisson, 2012). Extensive biochemical and physiological studies in *Gryllus* crickets have provided among the most detailed information on modifications of intermediary (e.g., lipid, amino-acid) metabolism and endocrine regulation that underlie life history specialization during adulthood (Table 1). However, endocrine

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Table 1
Morphological, physiological and life-history differences among the flight-capable [(LW(f)], the obligate flightless (SW) and the plastic-flightless [LW(h)] morphs of *G. firmus* during adulthood.

Trait	Morph			References
	LW(f)	SW	LW(h)[1]	
Wings	Fully developed	Short	Fully developed	Zera et al., 1997
Flight muscles	Fully developed; functional	Underdeveloped; non-functional	Histolyzed; non-functional	Zera and Denno, 1997; Zera et al., 1997
Capable of flight	Yes	No	No	Zera et al., 1997
Ovarian mass (day 5)	Small	Large (ca. 3–4-fold greater than LW (f))	Large – same as SW	Zera et al., 1997; Zera and Larsen, 2001; Zera, 2005
Lipid levels	High	Low	Low	Zera and Larsen, 2001; Zera, 2005
Lipid biosynthesis	High	Low	Low	Zhao and Zera, 2002; Zera, 2005; Zera and Harshman, 2009, 2011
Ovarian protein biosynthesis	Low	High	High	Zera and Zhao, 2006
Amplitude of JH titer cycle	Large	Small	Small	Zhao and Zera, 2004; Zera et al., 2007; Zera, 2016
Blood ecdysteroid level	Low	High	High	Zera and Bottsford, 2001; Zera et al., 2007

[1] The LW(h) morph is derived from the LW(f) morph by histolysis of flight muscles. Starting on day 3 of adulthood, an increasing frequency of LW(f) individuals are converted to the LW(h) morph as crickets age (Zera et al. 1997).

and biochemical studies undertaken to date in *Gryllus*, while detailed, have primarily focused on one important hormone, juvenile hormone (JH), and on a few pathways of metabolism. Numerous other hormones, metabolic regulators, and enzymes of intermediary metabolism likely contribute significantly to life history adaptation, but have yet to be investigated in *Gryllus*.

The endocrine regulation of life history trait expression in adult *Gryllus* morphs has recently become an especially intriguing topic of investigation. Since the 1960s, a widely held view has been that the insect hormone JH is a major regulator of morphological and life history differences between morphs of species exhibiting wing polymorphism, as well as other important polymorphisms/polyphenisms (Nijhout, 1994; Zera and Denno, 1997). However, recent detailed endocrine studies have called this hypothesis into question in *Gryllus*. The blood concentration of JH is now known to exhibit a dramatic circadian rhythm (> 10-fold change over several hours) in the dispersing morph of *G. firmus*, rising above and falling below the relatively constant titer in the flightless morph during the first week of adulthood (Zhao and Zera, 2004; Zera et al., 2007, 2017; Fig. 1). JH is now thought to play a more specialized role regulating traits involved in morph-dependent diurnal dispersal (exclusively at night; Zera et al., 2007, 2017; see Section 4). Other as yet unidentified hormones are expected to play a more important role than JH as regulators of traits that differ chronically (consistently over days without daily cycles) between the morphs, such as ovarian growth and lipid biosynthesis. The identity of the hormonal regulators of these "chronic" traits is now a central unresolved issue in life history endocrinology in *Gryllus*.

The documentation of the morph-specific JH titer circadian cycle in adult *G. firmus* has also made this species a useful empirical model to investigate diurnal/circadian aspects of life history adaptation. The majority of traits exhibit circadian rhythms or diurnal change (i.e., daily change which may be regulated by an endogenous circadian clock or exogenous environment variables) (Dunlap et al., 2004), and are thus expected to play an important role in life history adaptation (Zera et al., 2007, 2017). However, with some notable exceptions (Connor and Gracey, 2011; Beaver et al., 2002; Goodspeed et al., 2012; Hemond and Vollmer, 2015), the extent and significance of daily cycles of life history traits and their physiological underpinnings remain understudied. This is particularly the case for intraspecific variation in diel change, especially in a life history context, which has only been studied thus far with respect to the morph-dependent, genetically-based circadian rhythm for the hemolymph JH titer in *G. firmus* (Zera et al., 2007). Because JH, and hormones in general, regulate the expression of numerous genes in organisms (Goodman and Cousson, 2012; Jindra et al., 2013), morph-specific daily rhythms in the expression of functionally-important genes might be common in *G. firmus*.

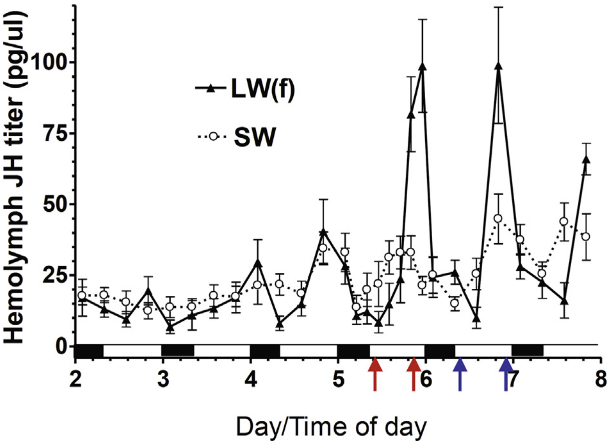

Fig. 1. Sampling schedule for RNA-seq and qRT-PCR experiments relative to the morph-specific JH titer circadian rhythm in flight-capable [LW(f)] and flightless (SW) morphs of *G. firmus*. Two arrows on day 5 (molt to adulthood = day 0) indicate the time of day that fat bodies were taken from LW(f) and SW morphs for transcriptome profiling (RNA-Seq). Samples for qRT-PCR measurement of transcript abundance on day 5 (same time as samples taken for RNA-Seq) and day 6 were taken at the time-of-day denoted by the four red or blue arrows. In addition (not shown above), samples for qRT-PCR developmental profiles (see Fig. 5) were taken on day 1 of adulthood. Dark bar = scotophase (lights-off); white bar = photophase (lights on). JH titer data are from Zhao and Zera (2004).

Recent global studies of gene expression have provided an enormous amount of new information on the functional causes of life history adaptation that can be used for subsequent, more detailed hypothesis testing (St-Cyr et al., 2008; Wheat et al., 2011; Savolainen et al., 2013; Jones et al., 2015; Kvist et al., 2015). We recently used RNA-Seq to construct a *de novo* flight muscle and fat body transcriptome for *G. firmus* (Vellichirammal et al., 2014). We then used this transcriptome to identify genes differing in transcript abundance between these organs and between the morphs of this species. However, because of restricted sampling (one time point, one pair of dispersing and flightless stocks), only limited conclusions could be drawn regarding the generality of chronic morph differences in gene expression, and no information was available on diel change in gene expression in the study of Vellichirammal et al. (2014)

The present study had two main goals. The first was to document morph-specific developmental profiles for the expression of genes