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The Endocrine Regulation of Wing Polymorphism in Insects: State of the Art, Recent Surprises, and Future Directions[1]

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Synopsis: The endocrine mechanisms controlling the development and reproduction of flight-capable (long-winged) and flightless (short-winged or wingless) morphs of wing-polymorphic insects have been intensively investigated. The "classical model," put forward in the early 1960s, postulates that morph-specific differences in development and reproduction are caused by variation in the titers of juvenile hormone (JH) and/or ecdysone. Despite decades of study, the importance of these hormones in regulating wing polymorphism in aphids and planthoppers remains uncertain. This uncertainly is largely a consequence of technical and size constraints which have severely limited the types of endocrine approaches that can be used in these insects. Recent studies in wing-polymorphic crickets (*Gryllus*) have provided the first direct evidence that the *in vivo* blood titers of juvenile hormone and ecdysone, and especially the activity of the JH regulator, juvenile hormone esterase, differ between nascent morphs. Morph differences are largely consistent with the classical model, although some types of data are problematic, and other explanations are possible. Adult morphs differ dramatically in the JH titer but titer differences are more complex than those proposed by the classical model. Detailed endocrine information is thus far available only for a few species of crickets, and the hormonal control of wing polymorphism for insects as a whole remains poorly understood. Future studies should continue to investigate the role of JH and ecdysteroids in morph development and reproduction, and should expand to include studies of morph-specific differences in hormone receptors and neurohormones.

Introduction

Many insects exhibit complex polymorphism, in which phenotypes (morphs) differ qualitatively and dramatically in a diverse array of traits and are specialized for functions such as flight, reproduction, defense, offense, or crypsis (Nijhout, 1994, 1999). These polymorphisms may result from a variety of causes: alternate morphs may be encoded by different genotypes (genetic polymorphism), induced by different environments (environmental polyphenism), or produced by variation in both genetic and environmental factors (referred to simply as polymorphism if the specific cause of morph production is not specified). Important examples include phase polyphenism in locusts, caste polyphenism in social insects, seasonal polyphenisms in butterflies, and dispersal (wing) polymorphism in a wide variety of insect groups (Fig. 1) (Hardie and Lees, 1985; Zera and Denno, 1997; Pener and Yerushalmi, 1998; Nijhout, 1994, 1999). These polymorphisms often play an integral role in the life cycle of the species in which they are found, for example, producing dispersing, or reproductive phenotypes adapted to a particular season or habitat. During the past two decades, complex polymorphism in insects has been intensively studied from ecological, evolutionary, physiological, and most recently biochemical and molecular perspectives (Nijhout and Wheeler, 1982; Hardie and Lees, 1985; Roff, 1986; Zera and Denno, 1997; Nijhout, 1999; Evans and Wheeler, 1999, 2001; Zera and Harshman, 2001; Zhao and Zera, 2002).

A central topic in the area of complex polymorphism, is the proximate endocrine processes that control morph development and reproduction. One of the most intensively studied complex polymorphisms from this perspective has been wing polymorphism. In this paper I will review work done on this topic during the past four decades, focusing primarily on studies undertaken in my laboratory on crickets of the genus *Gryllus*. More specifically, this review focuses on the following question, which has been at the heart of much of the endocrine research on wing polymorphism: "To what extent does modulation of the titers of juvenile hormone and ecdysone regulate developmental and reproductive characteristics of flight-capable and flightless morphs." Although the present paper is based on a talk given in a symposium on the physiological control of polyphenism, most of the endocrine work reviewed here deals with genetically-based wing polymorphism. This focus was necessitated since nearly all recent detailed endocrine work on wing polymorphism has been obtained from studies of genetically-based polymorphism. However, as discussed below, it is likely that broad aspects of endocrine control are similar for both environmentally-induced polyphenism and genetically-based polymorphism.

Background on Wing Polymorphism

Before discussing the endocrine regulation of wing polymorphism, it is essential to give some background information on the polymorphism itself. Dispersal and reproduction are energetically-expensive processes that commonly trade-

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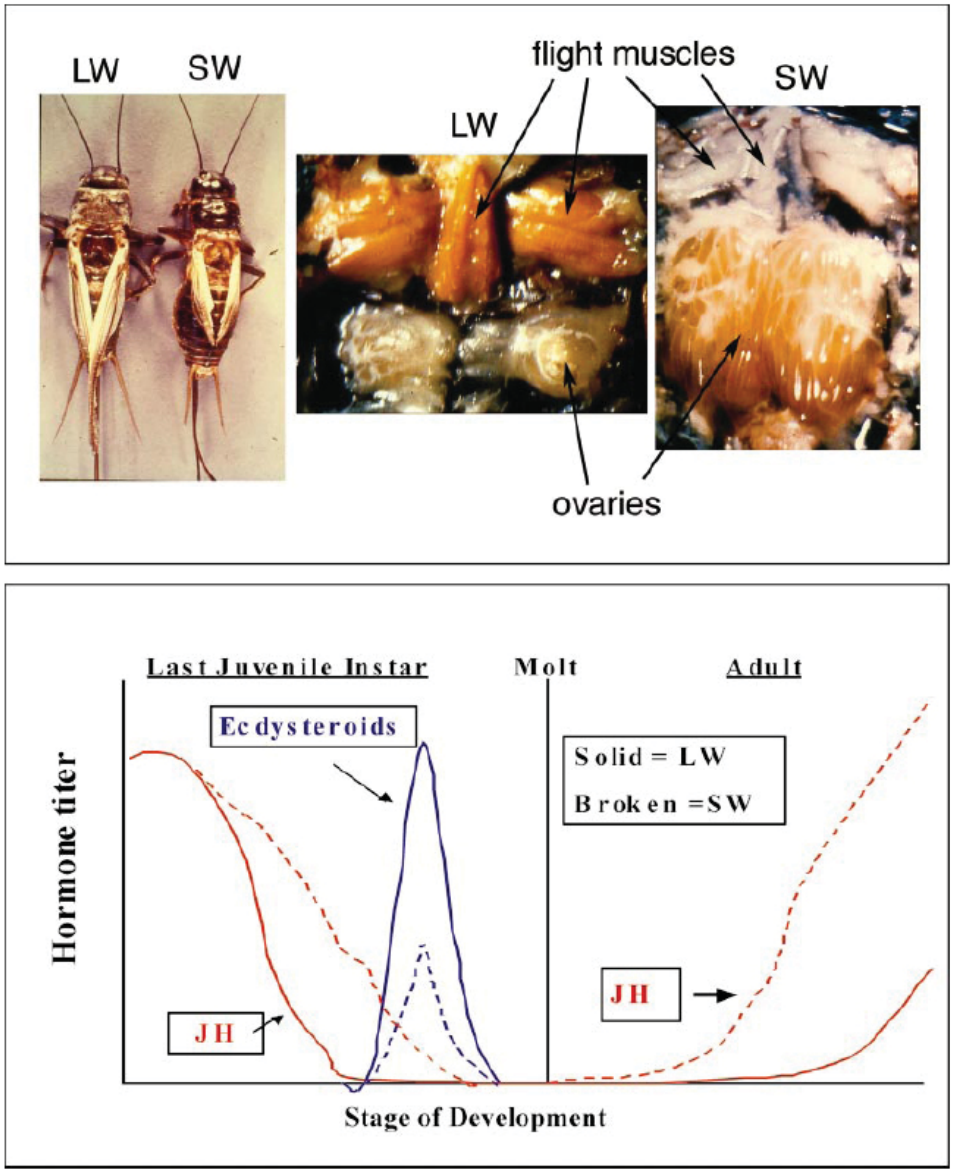

Figure 1. *Top panels:* Flight-capable (long-winged, LW) and flightless (short-winged, SW) female morphs of *Gryllus rubens* of the same age (day 5 of adulthood). In the top left panel, the fore wings have been removed to show variation in the hind wings. The middle and right panels illustrate dissections of morphs showing much larger and functional (pink) flight muscles, but much smaller ovaries in the LW *vs.* SW females. *Bottom panel:* The "classical model" of the endocrine control of wing morph development and reproduction. This panel illustrates hypothetical variation in the juvenile hormone (JH, red lines) and ecdysteroid (blue lines) titers that are thought to regulate differences in development and reproduction between LW and SW morphs.

off (*i.e.,* are negatively associated; Dingle, 1996; Zera and Denno, 1997; Zera and Harshman, 2001). The most dramatic expression of this trade-off is manifest as dispersal polymorphism in which qualitatively discrete phenotypes are produced, that are adapted for dispersal at the expense of reproduction and vice-versa (Fig. 1). The most extensively studied type of dispersal polymorphism, wing polymorphism, is common in many insect groups, most notably the Hemiptera/Homoptera (waterstriders, planthoppers and aphids), Coleoptera (beetles), and Orthoptera (crickets and grasshoppers) (Harrison, 1980; Roff, 1986; Masaki and Shimizu, 1995; Zera and Denno, 1997). While often referred to as "wing polymorphism," the polymorphism actually consists of morphs that differ in all major aspects of flight capability (*e.g.,* length of the wings, size of the flight muscles, production of flight fuels), as well as many aspects of reproduction (*e.g.,* onset and rate of ovarian growth). One morph has a fully-developed flight apparatus and is capable of flight but delays ovarian growth, while an alternate, obligately flightless morph has underdeveloped wings and flight

muscles, but begins reproducing earlier and has substantially-elevated early-age fecundity [Roff, 1986; Zera and Denno, 1997; for additional complications of flight muscle polymorphism (*i.e.,* variation in flight muscles but not wings), see Zera and Denno (1997) and Zera *et al.*, 1997]. In addition to these morphological and reproductive differences, a wide variety of biochemical, physiological, and behavioral traits differ between the morphs, and contribute importantly to morph-specialization for flight *vs.* reproduction (Tanaka, 1994; Sula *et al.*, 1998; Zera *et al.*, 1997, 1999; Zera and Denno, 1997; Zera and Harshman, 2001; Zhao and Zera, 2002). Depending upon the species, wing polymorphism may be exclusively a genetic polymorphism (*e.g.,* some beetles) or an environmental polyhenism (some aphids). Most commonly (*e.g.,* waterstriders, planthoppers, and crickets), the polymorphism results from variation in both genetic factors (typically polygenic control), and environmental factors (most commonly density, photoperiod, temperature, and food quality) (Roff and Fairbairn, 1991; Morooka and Tojo, 1992; Masaki and Shimizu, 1995; Zera and Denno, 1997). Ecological aspects of wing polymorphism, such as specific environmental factors that select for or against the flight-capable and flightless morphs in the field are reviewed in Vepsalainen (1978), Roff (1990), and Zera and Denno (1997**).**

Endocrine Control of Wing Polymorphism: The Classical Model

In the early 1960s insect physiologists and ecologists used newly obtained information on the endocrine control of metamorphosis to formulate an endocrine model of wing polymorphism (Wigglesworth, 1961; Southwood, 1961; Lees, 1966). This model has been the focus of experimentation and thinking about the hormonal mechanisms that regulate wing polymorphism up to the present (Lees, 1966; Gould, 1977; Nijhout and Wheeler, 1982; Hardie and Lees, 1985; Matsuda, 1987; Zera and Denno, 1997; Zera and Harshman, 2001). Although numerous hormones affect metamorphosis and reproduction, two hormones, ecdysone and juvenile hormone, are especially important in regulating these processes (Nijhout, 1994, 1999), and have been the almost exclusive foci of physiological studies of wing polymorphism.

Ecdysone has two important functions: it induces the molt and causes the expression of genes that specify adult characteristics (metamorphosis; Fig. 1). Juvenile hormone (JH), on the other hand, antagonizes the metamorphic action of ecdysone, while allowing the molt to occur. A high JH concentration causes a juvenile-to-juvenile molt, while a drop in the JH titer to a low or imperceptible level during the early last juvenile instar, allows ecdysone-induced gene expression to occur resulting in a metamorphic molt (*i.e.,* a molt from a juvenile to an adult in hemimetabolous insects [*e.g.,* crickets, aphids, waterstriders), or to a pupa in holometabolous insects (beetles, butterflies, moths, flies]). During the adult stage, JH, and/or ecdysone, takes on a new role as a gonadotropin, regulating, among other things, the synthesis of vitellogenin or yolk protein, and the uptake of these molecules into the developing oocyte. The hormonal regulation of metamorphosis, and reproduction, are, of course, much more complex than depicted in the brief outline given above. For example, many other hormones are involved in the regulation of these processes (Nijhout, 1994). Furthermore, temporal and tissue-specific expression of hormone receptors play critically-important roles in metamorphosis and reproduction, by defining the periods of time ("sensitive stages") during which tissues are sensitive to metamorphic or reproductive hormones (Nijhout, 1994, 1999).

The classical JH-wing-polymorphism model (Fig. 1) has focused primarily upon variation in the developmental timing, duration, and height of JH and ecdysteroid titer peaks, as the physiological causes of expression of morph-specific differences in morphology and reproduction. Variation in the expression of JH receptors has not been considered in this model, because the JH nuclear receptor has yet to be identified (see below). A variety of possible changes in the profiles of JH and ecdysone titers during development might underlie alternate morph development, only two of which are illustrated in Figure 1. The most common expectation of the classical model is that the JH titer should be higher in nascent short-wing destined individuals during some "sensitive" stage in development. In Figure 1, this is depicted as an elevated (delay in the decrease of) JH titer during the last juvenile stadium, sufficient to block the metamorphic effect of the rising ecdysteroid titer. Other possibilities would include an elevated JH titer during earlier instars or within the mother (in species with pre-natal morph determination), as proposed for aphids (Hardie and Lees, 1985). Alternatively, a reduced ecdysteroid titer (Fig. 1), a change in the timing of the ecdysteroid peak relative to the JH titer, or decreased expression of ecdysteroid receptors (not shown in Fig. 1) could result in ecdysteroid titers or tissue sensitivity that are insufficient to allow full growth and differentiation of wings and flight muscles, resulting in a short-winged morph with underdeveloped flight muscles. In adults, an earlier rise in the titer of JH (Fig. 1), the most widely studied gonadotropin in insects (Nijhout, 1994), has been the most common hypothesis put forward to account for the earlier ovarian growth of the flightless morph, which occurs in virtually all wing polymorphic species (Harrison, 1980; Roff, 1986; Zera and Denno, 1997).

Testing the Model: Aphids and Planthoppers

Aphids were the predominant model used during the 1960s, 70s and early 80s to investigate the endocrine control of wing polymorphism (mainly density-or photoperiodically-mediated wing polyphenism; Hales, 1976; Rankin and Singer, 1984; Hardie and Lees, 1985; Mittler, 1991; Hardie *et al.*, 1995; Zera and Denno, 1997). Only a brief summary of the aphid work will be given here since it has already been reviewed recently in a number of places (see above references). In a nutshell, despite decades of intensive work, the importance of JH

(or ecdysone) as a regulator of wing polymorphism in aphids remains uncertain. This uncertainty has resulted from a number of unfortunate technical problems that have severely limited studies of endocrine physiology in aphids. For example, the very small size of aphids has precluded the use of standard techniques such as the surgical removal of endocrine glands, or the *in vitro* measurement of juvenile hormone biosynthesis. The lack of identity of the major JH in this group also has substantially hampered measurement of the JH titer (Hardie and Lees, 1985; Hardie *et al.*, 1985; Zera and Denno, 1997).

Because of these limitations, workers have been forced to infer morph-regulating endocrine mechanisms almost exclusively from results of experiments involving application of exogenous hormones, agonists, and antagonists. However, only very limited conclusions can be drawn from such studies (Zera and Denno, 1997). For example, topical application of JH or JH analogues alters many *in vivo* endocrine traits, such as hormone titers and release of neuropeptides (*e.g.,* Smith and Nijhout, 1981; Stay *et al.*, 1994). Thus, in the absence of additional data, one can never be certain that an effect of an exogenous hormone is due to the applied hormone itself as opposed to an induced regulator. Furthermore no information on endocrine mechanisms (*e.g.,* do hormone titers differ between nascent morphs?) can be obtained from topical application experiments alone.

Thus, although JH, JH-agonists, and JH-antagonists (*e.g.,* precocenes) often affect adult morphology, interpretation of experimental results has been problematic (Rankin and Singer, 1984; Hardie and Lees, 1985; Mittler, 1991; Hardie *et al.*, 1995; Gao and Hardie, 1996; Zera and Denno, 1997). In many cases, JH affects on wing form appear to result from an abnormal disruption of metamorphosis (juvenilization) rather than induction of the apterous morph via a normal physiological mechanism. In other cases, JH, or JH agonists or antagonists, produce inconsistent effects, effects opposite those expected from the classical model, or no effects at all (Rankin and Singer, 1984; Hardie *et al.*, 1995; Gao and Hardie, 1996). The strongest case for a regulatory role for JH in aphid wing polymorphism is in photoperiodically-mediated wing polyphenism in *Aphis fabae* (Hardie, 1980; Hardie and Lees, 1985; Hardie *et al.*, 1995). However, it is unclear whether this is a special case (Hardie *et al.*, 1995), or whether JH itself is even involved given the uncertainties inherent in topical-application experiments discussed above. Similarly, a number of studies have shown that topical application of JH strongly redirects development from the long-winged to the short-winged morph, as well as enhancing ovarian growth in the brown planthopper, *Nilaparvata lugens* (Ayoade *et al.*, 1999; and references therein). Moreover, a JH antagonist (precocene II) induced formation of long-winged individuals in a genetic stock that normally produces short-winged individuals, and the effect of this antagonist could be obviated by simultaneous application of JH (Bertuso *et al.*, 2002). As is the case with aphids, the small size of planthoppers combined with uncertainties as to the major functional JH in this group has substantially hampered research on the endocrine control of wing polymorphism. Thus, the role of JH in wing polymorphism in planthoppers, while certainly suggestive, has yet to be firmly established, and no information is available on the endocrine mechanisms involved.

Testing the Classical Model: Crickets (*Gryllus*)

Morph-specific difference in endocrine titers and titer regulators

The endocrine control of wing polymorphism has been the most intensively studied in crickets of the genus *Gryllus*. Indeed, results of investigations over the past 15 years, provide some of the most detailed information on the endocrine mechanisms that potentially regulate morph-specific development for any case of complex polymorphism. In addition, recent studies on the JH titer in adult female morphs have yielded unexpected results which indicate that the endocrine control of morph-specific traits is more complex that previously suspected (Zera and Cisper, 2001; Zhao, and Zera, 2004). Advantages in using species of *Gryllus* as a model in endocrine studies of wing polymorphism include their large size, which allows quantification of hormone titers and titer regulators in the hemolymph (blood), various surgical manipulations, and *in vivo* studies of hormone metabolism (discussed below). Furthermore, the specific JH in crickets (and Orthoptera in general; Nijhout, 1994) has been identified, and several sensitive and specific radioimmunoassays have been developed for this JH (Zera *et al.*, 1989; Zera and Tobe, 1990; Goodman *et al.*, 1993; Zera and Cisper, 2001).

Endocrine studies have been undertaken on genetic stocks of two cricket species (*Gryllus rubens* and *G. firmus*) that produce primarily (>85%) flight-capable or flightless morphs under standard rearing conditions. Shifting presumptive long-winged (LW) *G. rubens* from standard to high densities as late as the penultimate and early last juvenile instar redirected development to the flightless, short-winged (SW) morph. Juvenile hormone applied during this time to nascent LW individuals had the same SW-promoting effect in *Gryllus rubens* (Zera and Tiebel, 1988) and *Modicogryllus confirmatus* **(**Zera and Tanaka, 1996). These results suggested that development of alternate morphs may be regulated by modulation of the JH titer/receptors during the last two juvenile instars.

Possibly the most important finding with respect to the endocrine control of wing polymorphism in *Gryllus* was the dramatic (3-to 6-fold) elevation in the hemolymph activity of juvenile hormone esterase (JHE) during the last juvenile instar in nascent LW *vs.* SW morphs in two *Gryllus* species, (Zera and Tiebel, 1989; Roff *et al.*, 1997; Zera and Huang, 1999; Fig. 2), and to a lesser degree in *Modicogryllus confirmatus* (Zera and Tanaka, 1996). JHE degrades juvenile hormone and is thought to regulate its titer in a number of insects (Hammock, 1985; Wyatt and Davey, 1996; Roe and Venkatesh,

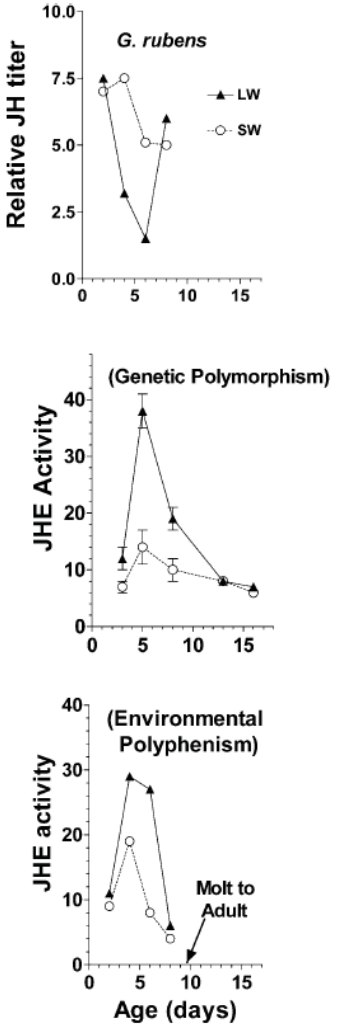

Figure 2. (*Upper and middle panels*) Differences in the developmental profiles of the hemolymph juvenile hormone titer (nM) and activity of juvenile hormone esterase (nmol JH-acid min^{-1} ml $hemolymph^{-1}$) between LW and SW genetic stocks of *G. rubens*. (*Lower panel*) JHE activities in LW and SW morphs produced from the LW stock when reared under different densities. Age refers to days since molt to the last juvenile instar. Data are from Zera *et al.* (1989) and Zera and Huang (1999).

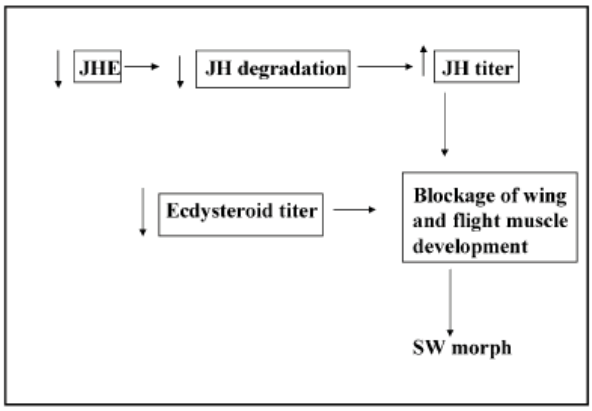

Figure 3. Endocrine model illustrating the regulation of development of LW and SW morphs of *Gryllus* species by modulation of juvenile hormone esterase activity, the juvenile hormone titer, and the ecdysteroid titer. See text for explanation.

1990). The activity of JH-epoxide hydrolase, which also degrades JH in parts of an insect other than hemolymph, did not differ between morphs of *G. firmus* (Zera and Huang, 1999).

Several pieces of evidence strongly suggest that hemolymph JHE regulates the development of LW and SW morphs in *Gryllus* (Figs. 2 and 3). First, as expected from the classical JH-wing-morph hypothesis, JHE activity is substantially reduced in the nascent SW morph, which is expected to result in an elevated JH titer in that morph (Zera and Huang, 1999). Moreover, reduced JHE activity occurred during the same period of time during which topical application of JH redirected development from the LW to the SW morph. Second, high JHE activity exhibits a nearly perfect co-segregation with long wings (wing morph) in crosses and backcrosses between LW and SW lines of two *Gryllus* species (Zera and Tiebel, 1989; A. Zera, unpublished data). Indeed, hemolymph JHE activity exhibits the tightest correlation with wing morph for any endocrine regulator investigated thus far. Third, morph-specific differences in JHE activity are positively correlated with morph-specific differences in *in vivo* JH degradation (Zera and Huang, 1999). Thus, variation in JHE activity results in variation in *in vivo* JH metabolism, a necessary prerequisite for JHE to affect the *in vivo* JH titer and morph development.

Thus far, the *in vivo* JH titer has been compared between nascent flight-capable and flightless morphs of only one *Gryllus* species, *G. rubens* (Zera *et al.*, 1989). No difference in the hemolymph JH titer was observed between morphs during the penultimate stadium. However, consistent with the JH-wing-morph hypothesis, a slightly elevated JH titer was found in nascent SW *vs.* LW morphs during the last stadium (Fig. 2; Zera *et al.*, 1989). Importantly, a reciprocal relationship was observed between the median hemolymph JH titer (higher in SW) and median hemolymph JHE activity (lower in SW; Fig. 2), consistent with the hypothesis that differences between morphs in the JH titer result from differences in JHE activity.

Despite this striking reciprocal relationship, only small differences in the JH titer have thus far been observed between nascent morphs during the last juvenile instar (Fig. 2). Titer differences between morphs may have been underestimated because of the large experimental error associated with JH titer measurements during the last juvenile stadium, when JH levels are very low (see discussion in Zera *et al.*, 1989). Alternatively, since the JH titer affects many aspects of metamorphosis, there may be a severe constraint on the degree to which the JH titer can differ between the morphs, even if this hormone is an important regulator of morph development. In amphibians, a low thyroxine titer is functionally important since it induces expression of the thyroxine receptor (Tata, 1996). Thus, only subtle JH titer differences could be functionally important by inducing the differential expression of JH receptors in nascent LW and SW morphs.

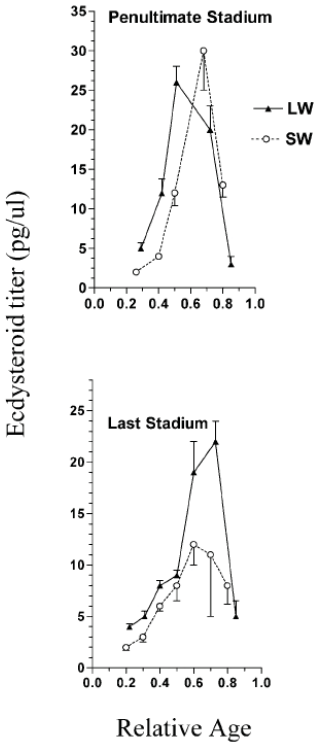

Figure 4. Differences in the hemolymph ecdysteroid titer between LW and SW morphs of *G. rubens* during the penultimate and last juvenile instars. Note the delayed rise in the ecdysteroid titer during the penultimate instar and lower ecdysteroid titer during the last juvenile instar in nascent SW *vs.* LW morphs. Data are from Zera *et al.* (1989).

Furthermore, alternate morph development may be regulated by combined differences in the titers of several hormones, only one of which is JH (see below). Finally, the subtle differences in the JH titer between morphs may be spurious and without functional importance.

Interestingly, morphs of *G. rubens* differed to a much greater degree in characteristics of the ecdysteroid titer than the JH titer (Zera *et al.*, 1989; Fig. 4). During the penultimate stadium, the ecdysteroid titer rose later and remained elevated for a shorter period of time in the SW compared with the LW morph. No difference was observed between the morphs in the height of the ecdysteroid peak. By contrast, during the last stadium, the timing of the rise, and the duration of elevation of the ecdysteroid titer were similar in both morphs. However, the height of the ecdysteroid titer peak was substantially lower in the SW morph. These various differences in the ecdysteroid titer are consistent with a role for ecdysteroids in morph determination (see above). Alternatively, the combined effect of an elevated JH titer and a reduced ecdysteroid titer during the last juvenile instar may be the key factor that inhibits wing and flight-muscle development in the SW morph, rather than variation in the titer of either hormone alone. At present, no information is available on morph-specific differences in tissue or developmental expression of ecdysteroid receptors. Since the ecdysteroid receptor has been cloned in a number of insects (see below), such a study would be feasible and highly informative in *Gryllus*.

Relationship between mechanisms regulating polymorphism and polyphenism

Although many species exhibit both genetically-specified polymorphism and environmentally-induced polyphenism, few data exist on the relationship between endocrine mechanisms controlling these two types of polymorphism (*e.g.,* Zera *et al.*, 1989; Roundtree and Nijhout, 1995*a*, *b*; Evans and Wheeler, 2001). In *G. rubens*, individuals from a LW-selected line were either reared under standard density (producing primarily the LW morph) or under higher density, producing the SW morph. JHE activity profiles differed between these wing-polyphenic morphs from the same genetic stock in a similar manner to profiles between LW individuals from a LW-selected line and SW individuals from a SW-selected line raised under the same environmental conditions (Fig. 2). Thus, the endocrine mechanisms that underlie genetically-specified and environmentally-induced wing polymorphism in *Gryllus* appear to share some common components.

Physiological, biochemical, and molecular causes of JHE activity differences between morphs

As a step towards identifying specific genes involved in the regulation of JHE and wing polymorphism in *Gryllus*, JHE enzymes from LW and SW morphs of *G. rubens*, or from genetic stocks of the congener *G. assimilis* selected for high or low JHE activity, were compared. The JHEs did not differ in any biochemical characteristic such as Michaelis constant, inhibition, or thermostability (Zera *et al.*, 1992; Zera and Zeisset, 1996). These and additional physiological studies (Zera and Huang, 1999) point to variation in as yet unidentified loci that regulate the concentration of the JHE enzyme, and the degree to which it is exported into the hemolymph, as the most likely cause of the substantial differences in hemolymph JHE activity between LW and SW morphs. The JHE from *G. assimilis* has been purified to homogeneity (Zera *et al.*, 2002), and a nearly full-length cDNA from *G. assimilis* has been isolated and sequenced (A. Zera, E. Crone, R. Russell, J. Oakeshott, unpublished). This JHE cDNA will now allow molecular-genetic studies on JHE and wing morph development to be undertaken in *Gryllus*.

JH titer differences between adult morphs

The first direct comparison of JH titers between adult morphs of a wing-polymorphic insect was undertaken in *G. firmus* (Zera and Cisper, 2001). Because the JH titer is much higher in adults than in juveniles, titers can be measured in single individuals and with much greater accuracy. Ovaries grow considerably faster (100–400%; Fig. 5, bottom panel) during the first week of adulthood in SW *vs.* LW *G. firmus* (Zera and Cisper, 2001; Zera and Bottsford, 2001). Since JH is a major gonadotropin in orthopterans such as *Gryllus* (Nij-

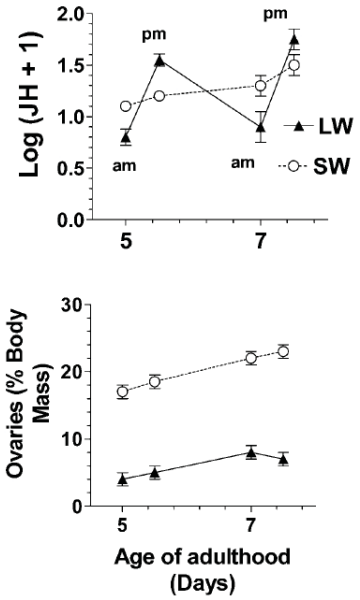

Figure 5. Differences in the hemolymph juvenile hormone titer (pg/ul) and ovarian mass between *adult* LW and SW *G. firmus*. Note the cyclic JH titer in LW females but temporally constant titer in SW females. Data are from Zera and Cisper (2001).

hout, 1994), the classic JH-wing-morph model predicts that the JH titer should rise earlier in the SW *vs.* the LW morph. Consistent with this expectation, topical application of JH or JH analogues to LW females of *G. firmus*, or other cricket species, during early adulthood, caused them to increase ovarian growth to a level seen in SW females (Tanaka, 1994; Zera and Cisper, 2001; A. J. Zera, unpublished)

However, direct titer measurements have revealed a much more complex and interesting situation: the JH titer rises 10–100 fold during the photophase in the flight-capable morph (LW with functional flight muscles), but is temporally constant in the SW morph (Fig. 5, top panel; Zera and Cisper, 2001; Zhao and Zera, 2004). This morph-specific diurnal change in the JH titer results in the titer being higher in the SW *vs.* LW morph during the early part of the photophase, but higher in the LW morph during the late photophase (Fig. 5; Zera and Cisper, 2001). The substantial, morph-specific diurnal cycle in the JH titer appears to be specific for JH, since comparable morph-specific changes were not seen for the ecdysteroid titer (Zhao and Zera, 2004).

These findings raise intriguing questions concerning the functional significance of the morph-specific patterns of diurnal change *vs.* constancy in the JH titer, and the proximate mechanisms involved. Our current working hypothesis is that the daily rise in the JH titer in the LW morph activates (*sensu* Elekonich and Robinson, 2000) flight behavior in that morph. This hypothesis is supported by the following. First, the diurnal change only occurs in the flight-capable morph, and begins on day 4 of adulthood (Zhao and Zera, 2004), when the LW morph first attains flight ability (Zera *et al.*, 1999). Second, as is the case with many other wing polymorphic insects, some LW adult *G. firmus* histolyze their flight muscles and become flightless (Zera *et al.*, 1997). Loss of the daily cyclicity in the JH titer occurs co-incident with flight muscle histolysis in the LW morph (Zera and Cisper, 2001). Finally, topical application of JH or JH analogues to many insects increases flight propensity (Rankin, 1991).

The hypothesis discussed in the preceding paragraph raises an interesting paradox. Since experimental elevation of the JH titer in *Gryllus* (and many other species) causes histolysis of flight muscles and ovarian growth (Zera and Cisper, 2001), why does the daily rise in the JH titer in the LW morph, which is several-fold higher than that in the SW morph, not cause flight muscle histolysis and initiate ovarian growth in the LW morph? There are several possible solutions to this paradox which are currently under study. First, the rise in the JH titer in the LW morph may be of sufficient duration to release flight behavior but of insufficient duration to initiate flight-muscle histolysis and ovarian growth. If this is the case, then the differential expression of morph-specific traits may be regulated by a novel mechanism: variation in the length of time required for an elevated titer of a single hormone to affect the expression of antagonistic traits. Alternatively, counteracting production of JH inhibitors, or down-regulation of JH receptors may occur in the LW morph, which may obviate the effects of the increased JH titer on certain tissues. Whatever the mechanisms involved, the endocrine regulation of morph-specific traits in adults is clearly more complex than envisioned by the classical model. Importantly, these unexpected JH titer differences between morphs could only have been identified by direct *in vivo* titer measurements. Not only would these titer differences have been completely missed if studies had only consisted of topical hormone application, we would have erroneously inferred that the JH titer was consistently higher in SW *vs.* LW females.

Summary, Synthesis, and Future Directions

During the past decade, considerable progress has been made in identifying the endocrine mechanisms that regulate wing polymorphism. However, because of the complexity of this problem, only the broad outlines of *potential* regulatory mechanisms can be seen at present. Most importantly, the first direct quantification of hemolymph hormone titers, and activities of titer regulators (juvenile hormone esterase) have been obtained for juvenile and adult morphs of a wing polymorphic insect.

In some cases, results of endocrine studies in *Gryllus* are consistent with expectations of the "classical model" (Fig. 1). Most notable is the very strong correlation between reduced activity of juvenile hormone esterase (JHE) during the last stadium in nascent SW individuals in two wing-polymorphic cricket species. A wide variety of data are consistent with the hypothesis that reduced JHE activity specifies the development of the SW morph (Figs. 2 and 3). Indeed, studies of JHE activity in wing-polymorphic crickets represent one of the most detailed investigations of the role of a specific endocrine regulator in morph development for any case of complex polymorphism in insects. The ecdysteroid titer has also

been implicated in regulating morph development in *Gryllus*, either in concert with, or independent of JH (Figs. 3 and 4).

Ironically, the weak link in the JHE-JH-wing-morph hypothesis in *Gryllus*, is the JH titer itself, which differs only subtly between morphs. As discussed above, there are reasons to expect that only small differences in the JH titer between nascent morphs during the last juvenile instar are possible and that these titer differences are functionally important. On the other hand, alternate explanations are possible. For example, it is conceivable that JHE might influence morph development by metabolizing a non-JH regulator, which would explain why JHE activity is strongly associated with wing morph, while the JH titer is not. Numerous JH-like compounds of unknown function exist in insects (Darrouzet *et al.*, 1998; Davey, 2000), and a diverse array of lipid molecules are involved in cell signaling (Karp, 2000). Purified JHE from *G. assimilis*, exhibits high apparent affinity for esters whose structures are very different from JH as well as for JH-III itself (Zera *et al.*, 2002). Furthermore, in some species, JH-acid, rather than being an inactive metabolite, may be an active hormone that is required for metamorphosis (*i.e.,* JH is a prohormone that is converted into an active hormone by JHE; Ismail *et al.*, 1998, 2000). If this were the case in *Gryllus*, it also might explain why JHE activity is more strongly associated with wing morph than is JH. These examples illustrate the point that even fundamental aspects of the endocrine regulation of morph development in *Gryllus* are not yet firmly established and may be very different than those originally envisioned. Indeed, the first direct measures of the JH titer in *adult* wing morphs (*G. firmus*; Zera and Cisper, 2001) have provided results very different from those expected from the "classical" model.

Despite important advances discussed above, the endocrine mechanisms that regulate wing polymorphism in insects as a whole remain largely an open issue. Hormone titers and titer regulators have only been measured in two cricket species, and even these basic pieces of endocrine information are lacking in aphids and planthoppers, despite decades of study. Many central aspects of morph regulation remain completely unstudied (*e.g.,* hormone receptors; see below). The situation for wing polymorphism is similar to that of phase polymorphism in locusts, the endocrine regulation of which is also still not well understood, even after many decades of intensive study (Pener and Yerushalmi, 1998). Indeed, only very recently have detailed measures of the JH titer been reported for adult locust phases (Tawfik *et al.*, 2000), and comparable JH titer data still are lacking for juvenile phases!

Future studies on the endocrine regulation of wing polymorphism should focus on four areas. First, basic studies on the role of juvenile hormone and ecdysteroids in regulating morph development and reproduction are still important given that direct measures of hormone titers and titer regulators are only available for a few cricket species. Second, no published information is available on morph-specific differences in any hormone receptor for any wing-polymorphic insect. Thus, a major aspect of the endocrine regulation of morph development and reproduction remains completely unstudied. Investigations of JH receptors in wing polymorphic insects must await the identification of the nuclear JH receptor in insects [see Truman and Riddiford (2002) for state-of-the-art in JH receptor studies]. On the other hand, the ecdyteroid receptor has been cloned in several insects (Fujiwara *et al.*, 1995; Riddiford *et al.*, 2001), including a wing polymorphic aphid (A. Pawlak-Skrzecz, G. N. Hannan, D. F. Hales and R. J. Hill, personal communication). During the next decade, a wealth of molecular information will almost certainly become available on morph-specific differences in hormone receptors, and other endocrine regulators (*e.g.,* juvenile hormone esterase; see above). Third, detailed investigations of the mechanisms by which hormones differentially regulate the development of target organs (*e.g.,* wings and flight muscles) are just beginning (*e.g.,* Kobayashi and Ishikawa, 1994; Nitsu, 2001) and will also likely expand considerably during the next decade.

Finally, studies of the endocrine regulation of wing polymorphism thus far have been narrowly focused on juvenile hormone and ecdysone. Numerous neurohormones regulate various aspects of reproduction and development in insects (Raabe, 1989; Nijhout, 1994; Bendena *et al.*, 1999). Locust phases differ in various neurohormones (Pener and Yerushalmi, 1998), and, numerous studies have implicated neurohormones in morph induction in aphids (Hardie and Lees, 1985; Zera and Denno, 1997). Neurohormonal regulation of morph development and reproduction will likely become one of the most prominent foci of future work on the endocrine control of wing polymorphism in insects.

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