August 1999

The Endocrine Genetics of Wing Polymorphism in *Gryllus*: Critique of Recent Studies and State of the Art

Anthony J. Zera
University of Nebraska - Lincoln, azera1@unl.edu

Published in *Evolution* 53:3 (1999), pp. 973–977.

Submitted May 6, 1998; accepted November 18, 1998.

COMMENTS

The Endocrine Genetics of Wing Polymorphism in *Gryllus*: Critique of Recent Studies and State of the Art

Anthony J. Zera

School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588

E-mail: azera1@unl.edu

Keywords: Genetic correlation, heritability, juvenile hormone, juvenile hormone esterase, liability, threshold model, wing polymorphism

In a series of papers, the most recent of which was published in *Evolution,* quantitative-genetic experiments were undertaken on reproductive and physiological correlates of wing polymorphism in the sand cricket, *Gryllus firmus* (Fairbairn 1994; Fairbairn and Yadlowski 1997; Roff et al. 1997). A goal of these studies was to determine the physiological causes underlying: (1) genetic variation for an ecologically important threshold trait (wing polymorphism); and (2) genetic correlations between wing morph and other reproductive and migratory features, such as fecundity and flight muscle histolysis. These authors concluded that genetic variation for the activity of the endocrine regulator, juvenile hormone esterase (JHE), is "causally related to the production of alternate morphs" in *G. firmus* (Roff et al. 1997, p. 1917). One of these studies (Fairbairn and Yadlowski 1997) has recently been elevated to a textbook example of the physiological mechanisms regulating the expression of quantitative-genetic variation (Schlichting and Pigliucci 1998).

I take issue with many of the conclusions concerning the endocrine basis of wing polymorphism stated in papers by Roff, Fairbairn, and coworkers (Fairbairn 1994; Fairbairn and Yadlowski 1997; Roff et al. 1997). These authors clearly have demonstrated a *correlation* between the activity of JHE during the last nymphal stadium and the subsequent molt to either a long-winged or short-winged adult. However, the studies lack any functional information on the role of JHE in regulating alternate morph development or reproduction in *G. firmus*. Therefore, their conclusions concerning a *causal* relationship between genetic variation for the activity of this enzyme and alternate wing morph development or reproduction in *G. firmus* are premature. Furthermore, these papers present an inaccurate account of data and discussion of the role of JHE in wing morph development in the congener, *Gryllus rubens,* reported by myself and colleagues (e.g., Zera and Tiebel 1989; Zera et al. 1989; Zera and Holtmeier 1992; Zera and Denno 1997). Accounts by Fairbairn and Yadlowski (1997) and Roff et al. (1997) give the impression that the regulatory role of JHE in wing morph development in *Gryllus* is understood to a much greater degree than is actually the case, and therefore are counterproductive to future research on this topic.

Wing polymorphism is becoming an increasingly useful model for investigating the physiological mechanisms underlying genetically based variation in morphology, development, and life-history (Roff 1986; Zera and Denno 1997; Zera et al. 1998). Future progress on this model is critically dependent upon an accurate assessment of which physiological hypotheses are well supported and thus can serve as strong foundations for subsequent research. The main goals of this commentary are: (1) to point out serious errors concerning the endocrine regulation of wing polymorphism in *Gryllus* that are contained in papers of Roff, Fairbairn, and colleagues; and (2) to establish which aspects of the endocrine regulation of wing polymorphism are well supported and which are not. I also highlight what I consider to be pitfalls and appropriate methods in the analysis of endocrine variation which I hope will be helpful to nonphysiologists. I will first review basic aspects of wing polymorphism and its endocrine regulation to provide background for the issues raised in this commentary.

Background on Wing Polymorphism and Its Regulation by Juvenile Hormone

Wing polymorphism occurs commonly in many of the major insect orders and has been used extensively during the past 50 years as a model to study the evolution of dispersal and life histories (Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). The polymorphism consists of discontinuous variation in a diverse set of anatomical, physiological, and life-history traits resulting in morphs that are specialized for dispersal or reproduction. For example, wings and flight muscles are fully developed in the flight-capable, long-winged (LW) morph but are substantially underdeveloped in the flightless short-winged (SW) or wingless morph. Importantly, flight capability trades-off with reproductive effort: SW females begin ovarian growth at an earlier age and often exhibit greater overall fecundity than their LW counterparts.

A long-standing issue in wing polymorphism is the physiological mechanisms which control developmental and reproductive aspects of the LW and SW morphs. The most widely discussed hypothesis focuses on juvenile hormone as a key regulator of these features (Southwood 1961; Wigglesworth 1961; Roff 1986; Zera and Denno 1997). In insects, juvenile hormone, above some threshold level during a sensitive period in the juvenile molt cycle, causes the retention of juvenile features resulting in a molt to another juvenile stage (stadium) (Nijhout 1994). During the early-mid last juvenile stadium, the juvenile hormone titer drops to a very low or undetectable level. A surge of ecdysone (molting hormone) during this time, causes a metamorphic

molt in which the juvenile is transformed into an adult (hemimetabolous insects such as crickets, grasshoppers, aphids), or a pupa (holometabolous insects such as flies, moths, butterflies). In most adults, juvenile hormone regulates many aspects of reproduction such as the synthesis of vitellogenin (yolk protein) and uptake of vitellogenin into eggs (Wyatt and Davey 1996).

The juvenile hormone-wing morph hypothesis (Southwood 1961; Wigglesworth 1961) posits that an elevated juvenile hormone titer during development blocks the full growth and differentiation of wings and flight muscles resulting in a SW (or wingless) morph. An increased level of juvenile hormone in SW adults purportedly causes the earlier ovarian growth in that morph (Zera and Denno 1997).

Thus far, the only direct test of the juvenile hormone-wing morph hypothesis has been undertaken in the cricket *G. rubens* (summarized in Zera and Denno 1997). Experimental elevation of the juvenile hormone titer during the last stadium in LW-destined *G. rubens* redirected their development to the SW form. This suggests that juvenile hormone is involved in morph determination (Zera and Tiebel 1988). Activity of juvenile hormone esterase, an enzyme which degrades and regulates juvenile hormone in many insects (Hammock 1985; Roe and Venkatesh 1990), was much higher in LW versus SW selected-lines, and high JHE activity strongly cosegregated with the LW morph in interstock crosses (Zera and Tiebel 1989). Furthermore, high JHE activity was associated with elevated *in vivo* juvenile hormone degradation and a slightly reduced juvenile hormone titer in nascent LW individuals (Zera et al. 1989; Zera and Holtmeier 1992). All of these data are consistent with the hypothesis that reduced JHE activity causes reduced JH catabolism leading to a delay in the reduction of the JH titer which, in turn, gives rise to a SW morph. However, differences in the *in vivo* JH titer between nascent LW and SW morphs of *G. rubens* during the last stadium are not large. It is presently unclear whether these differences are functionally important with respect to regulating wing morph development (Zera et al. 1989; Zera and Denno 1997). Finally, there are many endocrine factors other than juvenile hormone that could play a major role in regulating wing morph development and the influence of these factors has barely been investigated (Zera and Denno 1997). In one of the few studies of these hormones, Zera et al. (1989) found that ecdysteroid levels varied between LW and SW morphs of *G. rubens* to a much greater degree than juvenile hormone levels. This suggests that ecdysteroids may play the primary role in regulating morph development in *G. rubens.* To summarize, although several pieces of evidence support a role for JHE in regulating wing morph development in *G. rubens,* such a role has yet to be firmly established in this species (Zera et al. 1989; Zera and Denno 1997; see below). As will be discussed in detail below, the functional role of JHE in regulating wing morph development in other species of *Gryllus* has barely been investigated.

Critique of Studies on JHE and Wing Morph Induction in G. firmus by Roff, Fairbairn, and Co-authors

Roff, Fairbairn, and co-authors (Roff et al. 1997; Fairbairn and Yadlowski 1997) measured hemolymph JHE activities in half-sib families and in LW and SW-selected lines of the congener, *G. firmus.* In these papers, a number of strong claims were made concerning the causal role of JHE and juvenile hormone in wing morph determination in this species. The following are a few examples (italics within these quotes are mine): "Despite the possible role of other aspects of JH metabolism and other hormones, the strong correlated response of JHE to selection on wing morphology *demonstrates* its importance in the regulation of wing dimorphism" (Discussion of Fairbairn and Yadlowski 1997). "In addition to the shift in mean JHE activity, we found a change in the relationship between JHE and morph induction: *the level of JHE that induces wing production* changed in response to selection" (Discussion of Fairbairn and Yadlowski 1997). "This study and that of Fairbairn and Yadlowski (1997) together demonstrate that JHE activity is both phenotypically and genetically correlated with wing dimorphism in *G. firmus.* This is the first demonstration of a genetic and thus evolutionary important basis in a physiological trait that is *undoubtedly causally related* to the production of alternate morphs." (Roff et al. 1997, p. 1917).

There are many problems with these claims. First, the only evidence provided by Fairbairn, Roff, and co-authors that different JHE activities cause the production of alternate wing morphs in *G. firmus* is the correlation between high JHE activity during the last juvenile stadium and the subsequent molt to the long-wing morph. This type of purely correlational data is clearly insufficient to establish a causal connection between these two traits. The argument of Roff, Fairbairn, and co-authors is based on the untested assumption that elevated blood JHE activity, measured *in vitro*, causes elevated JH catabolism, *in vivo*, which in turn causes a reduced JH titer which regulates the production of LW *G. firmus.* The danger in making such assumptions can be seen in lines of the congener, *G. assimilis,* that have been selected for elevated versus decreased hemolymph JHE activity (Zera and Zhang 1995). By the sixth generation of selection, JHE activities differed between lines of *G. assimilis* to a greater degree than did JHE activities between LW and SW selected lines of *G. firmus* studied by Fairbairn and Yadlowski (1997). However, in two of three blocks (replicate selection trials), *in vivo* juvenile hormone degradation was virtually identical in the high and low activity lines of *G. assimilis* (Zera and Zhang 1995). Continued selection on *G. assimilis* did result in differences in both JHE activity and juvenile hormone degradation (Zera et al. 1996). Nevertheless, the results of Zera and Zhang (1995) clearly show that substantial differences in JHE activity between genetic stocks do not necessarily result in differences in juvenile hormone catabolism. The bottom line here is that physiological consequences of enzyme activity variation must be directly established rather than assumed. This is one of the main lessons of two decades of allozyme research. In some cases, kinetic differences between allozymes clearly result in measurable differences in physiology (Hilbish et al. 1982; Burton and Feldman 1983; Watt 1994). However, in other cases, a 50% or greater reduction in enzyme activity may have no demonstrable effect on pathway flux (fig. 1 of Kacser and Burns 1981; fig. 5 of Dykhuizen and Dean 1990).

Second, Roff, Fairbairn, and co-authors have not provided any evidence that the juvenile hormone itself has any effect

on wing morph development. Clearly, these authors must establish that juvenile hormone itself regulates wing morph development if they are to make a strong case that JHE affects morph development by modulating the juvenile hormone titer. A variety of approaches are available to establish the function of a particular hormone. One necessary (but not sufficient) procedure, which is relatively simple to perform, and which has been used extensively in endocrine studies of wing polymorphism, is to experimentally alter the *in vivo* titer of the hormone in question (e.g., by topical application of JH). One then determines if this treatment produces some predicted effect (e.g., causes presumptive LW individuals to molt into SW adults) (Hardie and Lees 1985; Zera and Tiebel 1988; Dingle and Winchell 1997; Zera and Denno 1997). Roff, Fairbairn, and co-authors did not perform this simple hormone manipulation experiment or any other procedure which would provide even preliminary evidence that juvenile hormone regulates wing morph development. The importance of obtaining at least some preliminary information on the role of juvenile hormone itself in wing morph determination is underscored by the many studies that have failed to obtain expected results from juvenile hormone manipulations (Hardie and Lees 1985; Zera and Denno 1997). Assumptions about the role of juvenile hormone in regulating wing morph development often have proven to be incorrect and hence need to be tested experimentally *for each new species under consideration.* Indeed, Dingle and Winchell (1997) have reported that genetic stocks (families) of the same species differ in their responsiveness to juvenile hormone, indicating that such tests may be required for each genetic stock under study.

Third, in the absence of any direct information on relationships among JHE activity, JH degradation, JH titer, and wing morph development in *G. firmus,* Roff, Fairbairn, and co-authors drew on data documenting such relationships in *G. rubens* (Zera and Tiebel 1989; Zera et al. 1989; Zera and Denno 1997) and argued that similar relationships also exist in *G. firmus.* This approach is seriously flawed. Alternate wing morph development could conceivably be regulated by variation in any of several mechanisms (synthesis, degradation, transport) that produce variation in the titer of any of several hormones (juvenile hormone, ecdysteroids, neurohormones; see Zera and Denno 1997). Thus far, virtually all published information on endocrine variation between wing morphs has come from studies of a single species, *G. rubens* (see references above). Given the paucity of information on endocrine mechanisms in *Gryllus,* it is clearly unjustified to argue, *a priori*, that endocrine-morph relationships which have been documented in one species will also exist in another species, for which no information is available.

Recent unpublished studies from my laboratory have, in fact, documented that associations between endocrine traits or associations between endocrine and whole organism features can vary dramatically between species. For example, although the ecdysteroid (molting hormone) titer is dramatically elevated in nascent LW versus SW morphs of *G. rubens* during the last stadium, and may regulate morph development in this species (Zera et al. 1989), no comparable ecdysteroid titer variation has been found between nascent morphs of *G. firmus* during this developmental stage (A. J. Zera, D. Katz, and J. Bottsford, unpubl. data). Furthermore, as mentioned above, selected lines of *G. assimilis* differ in hemolymph JHE activity to a similar or greater degree as do LW and SW *G. firmus* (Roff et al. 1997; Fairbairn and Yadlowski 1997). Yet these lines of *G. assimilis* did not differ in *in vivo* JH degradation (Zera and Zhang 1995; see above) and do not vary in wing length (A. Zera, unpubl. data).

Fourth, Roff, Fairbairn, and colleagues present an inaccurate account of published data and discussion on the role of JHE in wing morph development in *G. rubens*. Information provided in their papers makes it seem that a role for JHE in wing morph development is more firmly established in *G. rubens* than is actually the case. I have previously stated that the role of JHE in wing morph development remains tentative in *G. rubens* (Zera and Tiebel 1989; Zera et al. 1989; Zera and Denno 1997). For example, in the first study which reported JHE activity differences between LW and SW *G. rubens,* an entire paragraph was devoted to this point (see Zera and Tiebel 1989, p. 15). Similarly, the concluding paragraph of the Discussion of Zera et al. (1989) clearly states that JH titer variation is only subtle and that future work needs to be done "to determine whether the titre differences are correlated or causative factors in wing morph determination" (Zera et al. 1989). This is echoed in a recent review by Zera and Denno (1997, pp. 218–219) which states that "Although the endocrine data obtained for *G. rubens* are consistent with the classical JH-morph determination hypothesis, other interpretations cannot be ruled out. Most importantly, the JH titer differences between morphs are small, and thus their functional significance can be questioned." By contrast, Fairbairn and Yadlowski (1997) state without qualification (in referring to my studies of *G. rubens*) "the JH titre in this period is determined primarily by the activity of the degradative enzyme, juvenile hormone esterase," and that "wing morphology is strongly influenced by the JH titer during the final nymphal stadium." The fact that the role of JHE in wing morph determination remains uncertain in *G. rubens,* the only species in which this phenomenon has been studied in any detail, further undercuts the argument by Roff, Fairbairn, and colleagues that JHE plays an analogous role in *G. firmus,* a species for which there is no published data on JHE function.

Conclusions

Wing polymorphism is an attractive model for investigating the physiological mechanisms underlying genetically based variation in morphology, development, and life history (Zera and Denno 1997; Zera et al. 1998). However, future progress on this model depends upon a thorough understanding as to which physiological aspects of morph development and reproduction are well established and which are not. During the past decade, important advances have been made in documenting associations between key endocrine traits and wing morph in *G. rubens* (e.g., JHE activity, JH degradation, juvenile hormone titer, and ecdysteroid titer; summarized in Zera and Denno 1997) and in *G. firmus* (JHE activity: Fairbairn and Yadlowski 1997; Roff et al. 1997). These data are important because they are the first to identify specific potential regulators of wing morph development in any wing polymorphic insect. However, in contrast to statements made by Fairbairn, Roff, and colleagues (see above), we have a long way

to go before the functional significance of these endocrine-wing morph associations is well established.

There is a growing consensus among evolutionary biologists that a deep understanding of the evolution of quantitative-genetic variation requires knowledge of the proximate mechanisms that regulate the expression of phenotypic variation (Schlichting and Pigliucci 1998). Therefore, physiological studies of quantitative-genetic variation will almost certainly become more common in the near future. It is important that evolutionary biologists interested in this research topic appreciate the complex nature of endocrine interactions (Pener 1991; Nijhout 1994) and the difficulties involved in establishing the endocrine basis of phenotypic variation. The challenges involved in this task are nowhere more apparent than for the case of phase polymorphism in locusts. This topic has been the focus of research in insect endocrinology since the 1950s, yet the endocrine factors that regulate phase determination still remain illusive (Pener 1991). The complexity of endocrine mechanisms does not mean that hormonal aspects of phenotypic evolution are likely to be more difficult to investigate than other problems in evolutionary biology. For example, we only need to look at the immense effort that was required to document the adaptive basis of individual enzyme polymorphisms (Watt 1994; Koehn and Hilbish 1995). The point is that we should not have unrealistic expectations about the simplicity of hormonal mechanisms which regulate phenotypic expression. Specifically, workers should be careful to avoid postulating facile functional explanations for endocrine variation that are based on limited investigation. This approach will be counterproductive in the long run and will stifle progress in understanding evolutionary aspects of endocrine variation as it has stifled research on the proximate endocrine mechanisms regulating phase polymorphism in locusts (Pener 1991). One of the worst things that could happen to the fledgling subdiscipline of evolutionary endocrinology is that potential researchers are given the impression that certain key issues have been resolved, when, in fact, they remain poorly understood.

Acknowledgments

My research has been supported by grants from the National Science Foundation, the most recent of which are IBN- 9507388 and IBN-9808249. I thank Drs. Larry Harshman and Tony Joern, the reviewers, and the Corresponding Editor for their thoughtful comments on earlier drafts of this manuscript.

Literature Cited

Burton, R. S., and M. W. Feldman. 1983. Physiological effects of an allozyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod *Tigriopus californicus*. Biochem. Genet. 21 :239–251.

Dingle, H. 1996. Migration: the biology of life on the move. Oxford University Press, Oxford, U.K.

Dingle, H., and R. Winchell. 1997. Juvenile hormone as a mediator of plasticity in insect life histories. Arch. Insect Biochem. Physiol. 35:359–373.

Dykhuizen, D. E., and A. M. Dean. 1990. Enzyme activity and fitness: evolution in solution. Trends Ecol. Evol. 5:257–262.

Fairbairn, D. J. 1994. Wing dimorphism and the migratory syndrome: correlated traits for migratory tendency in wing dimorphic insects. Res. Popul. Ecol. 36:157–163.

Fairbairn, D. J., and D. E. Yadlowski. 1997. Coevolution of traits determining migratory tendency: correlated response of a critical enzyme, juvenile hormone esterase, to selection on wing morphology. J. Evol. Biol. 10:495–513.

Hammock, B. D. 1985. Regulation of the juvenile hormone titer: degradation. Pp. 431–472 *in* G. A. Kerkut and L. I. Gilbert, eds. Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 7, Pergamon Press, New York.

Hardie, J., and A. D. Lees. 1985. Endocrine control of polymorphism and polyphenism. Pp. 441–449 *in* G. A. Kerkut and L. I. Gilbert, eds. Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 8, Pergamon Press, New York.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annu. Rev. Ecol. Syst. 11:95–118.

Hilbish, T. J., L. E. Deaton, and R. K. Koehn. 1982. Effect of an enzyme polymorphism on regulation of cell volume. Nature 298:688–689.

Kascer, H., and J. A. Burnes. 1981. The molecular basis of dominance. Genetics 97:639–666.

Koehn, R. K., and T. J. Hilbish. 1995. The adaptive importance of genetic variation. Pp. 182-189 *in* M. Slatkin, ed. Exploring evolutionary biology. Readings from American scientist. Sinauer Associates, Sunderland, MA.

Nijhout, H. F. 1994. Insect hormones. Princeton Univ. Press, Princeton, NJ.

Pener, M. P. 1991. Locust phase polymorphism and its endocrine relations. Adv. Insect Physiol. 23:l–80.

Roe, R. M., and K. Venkatesh. 1990. Metabolism of juvenile hormones: degradation and titer regulation. Pp. 126–179 *in* A. P. Gupta, ed. Morphogenetic hormones of arthropods. Rutgers University Press, New Brunswick, NJ.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009–1020.

Roff, D. A., G. Stirling, and D. J. Fairbairn. 1997. The evolution of threshold traits: a quantitative genetic analysis of the physiological and life-history correlates of wing dimorphism in the sand cricket. Evolution 51:1910–1919.

Schlichting, C., and M. Pigliucci. 1998. Phenotypic evolution. Sinauer Associates, Sunderland, MA.

Southwood, T. R. E. 1961. A hormonal theory of the mechanism of wing polymorphism in *Heteroptera.* Proc. Roy. Ent. Soc. Lond. 36:63–66.

Watt, W. B. 1994. Allozymes in evolutionary genetics: self-imposed burden or extraordinary tool? Genetics 136:11–16.

Wigglesworth, V. B. 1961. Insect polymorphism—a tentative synthesis. Pp. 103–113 *in* J. S. Kennedy, ed. Insect polymorphism. Royal Entomological Society, London.

Wyatt, G. R., and K. G. Dayey. 1996. Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. Adv. Insect Physiol. 26:l–155.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42:207–231.

Zera, A. J., and C. L. Holtmeier. 1992. In vivo and in vitro degradation of juvenile hormone-III in presumptive long-winged and

short-winged *Gryllus rubens.* J. Insect Physiol. 38:61–74.

Zera , A. J., and K. C. Tiebel. 1988. Brachypterizing effect of group rearing, juvenile hormone-III, and methoprene on wing-length development in the wing-dimorphic cricket, *Gryllus rubens.* J. Insect Physiol. 34:489–498.

Zera , A. J. 1989. Differences in juvenile hormone esterase activity between presumptive macropterous and brachypterous *Gryllus rubens:* implications for the hormonal control of wing polymorphism. J. Insect Physiol. 35:7–17.

Zera, A. J., and C. Zhang. 1995. Direct and correlated responses to selection on hemolymph juvenile hormone esterase activity in *Gryllus assimilis.* Genetics 141:1125–1134.

Zera, A. J., C. Strambi, K. C. Tiebel, A. Strambi, and M. A. Rankin. 1989. Juvenile hormone and ecdysteroid titers during critical periods of wing morph determination in *Gryllus rubens.* J. Insect Physiol. 35:501–511.

Zera, A. J., J. Potts, and K. Kobus. 1998. The physiology of life history trade-offs: experimental analysis of a hormonally induced life-history trade-off in *Gryllus assimilis.* Am. Nat. 152:7–23

Zera, A. J., J. Sall, and R. Schwartz. 1996. Artificial selection on JHE activity in *Gryllus assimilis:* nature of activity differences. between lines and effect on JH binding and metabolism. Arch. Insect Biochem. Physiol. 32:421–428.

Corresponding editor for *Evolution*: C. Boggs

A response to this critique, by Daphne J. Fairbairn and Derek A. Roff, was published as the article immediately following in Evolution 53:3 (1999), pp. 977-979.