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# Adaptation, Exaptation, and Constraint: A Hormonal Perspective

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**ABSTRACT:** We approach conceptual issues in evolutionary biology from an endocrinological perspective, noting that single hormones typically act on several target tissues and thereby mediate suites of correlated phenotypic traits. When several components of such a suite are beneficial, an important evolutionary question is whether all are adaptations or some are exaptations. The answer may depend on whether the traits arose in response to selection on variation in systemic levels of the hormone or on variation in responsiveness of target tissues to invariant levels of the hormone. If the former, selection probably acted directly on fewer than all traits; beneficial traits arising indirectly would be exaptations. In contrast, multiple beneficial traits that arose out of independent changes in target-tissue sensitivity to invariant hormone levels could all be adaptations. Knowledge of specific hormonal mechanisms as well as of historical selective regimes will be necessary to draw such distinctions. Endocrine constraints on evolution can be studied experimentally by applying hormones systemically and measuring interdependent responses of beneficial and detrimental traits to selection (phenotypic engineering with hormones). Supposing that alteration of one trait in isolation would enhance fitness, cases in which the net effect of endocrine alteration of multiple traits is to depress fitness provide evidence for constraints. We briefly report results of recent studies employing hormonal manipulations, stressing our own work on the dark-eyed junco (*Junco hyemalis*: Emberizidae).

*Keywords:* testosterone, phenotypic engineering, trade-offs, dark-eyed junco, *Junco hyemalis*.

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Our purpose in this article is to discuss how descriptive and experimental studies of hormones can contribute to the resolution of issues of current interest in the study of adaptation. According to Rose and Lauder (1996), the study of adaptation is experiencing a "post-Panglossian renaissance." Having assimilated the lessons of Gould and

Lewontin (1979), evolutionary biologists who study adaptation have replaced presumptions of trait-by-trait perfection with more sophisticated views, informed by advances in theory, new phylogenies, studies of selection, and philosophical debate about the nature of adaptation (Rose and Lauder 1996).

One advance has been to achieve a measure of agreement about what is required to establish a feature of an organism as an adaptation. For example, Sheldon and Whittingham (1997, p. 285) recently offered the following composite definition, which they attributed to Gould and Vrba (1982), Coddington (1988, 1994), and Baum and Larson (1991; see also Lauder 1996):

An adaptation is an apomorphic feature that evolved in response to an apomorphic function (Coddington 1994). It has current utility and was generated historically through the action of natural selection for its current biological role (Baum and Larson 1991).

Under this definition, for traits to achieve the status of adaptation, they must be shown to be derived (apomorphic) by demonstrating that they appear in the phylogenetic record subsequent to an environmental change that made them advantageous (apomorphic function). They must also be established as advantageous in the present (have current utility) and as having arisen as the direct result of past selection for the function that they presently serve (they must not be exaptations; discussed later in this introduction).

Modern students of adaptation rely on advances in the study of phylogenetics and the comparative method to determine whether a trait is derived and, while controlling for common descent, to establish "good fit" between the trait and the environment (Brooks and McLennan 1991; Harvey and Pagel 1991). To determine whether and how attributes are advantageous in the present, they conduct studies of selection in action, and they establish a trait's utility through experimental manipulations (Endler 1986; Mitchell-Olds and Shaw 1987; Grafen 1988; Schluter 1988; Ketterson and Nolan 1992; Ketterson et al. 1996; Sinervo and Basolo 1996). When applied to the study of single

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traits, these methods have greatly improved our understanding of adaptation. Problems remain, however, especially with respect to multiple traits that are correlated in their expression. Our major objective is to suggest that resolution of problems involving such multiple, correlated traits will be advanced by study of the mechanisms that control trait expression, particularly hormonal mechanisms.

A first and widely recognized problem is that of distinguishing adaptations from exaptations. According to Reznick and Travis, "The current focus of the debate about natural selection lies in defining the cause and effect relationship between a trait that appears to be adaptive and the factors that selected for the evolution of the trait" (1996, p. 243). With correlated traits, establishing cause and effect is particularly challenging because not all the traits are necessarily adaptations. Some, while favorable, may have arisen not as the result of any benefit they provided at the time selection acted but as the result of their coexpression with traits that were acted on directly by the environment; that is, they may be exaptations (Gould and Vrba 1982). Relatively little progress has been made in determining how, operationally, to distinguish adaptations from exaptations (Curio 1973; West-Eberhard 1992*a*). Because hormones typically affect whole suites of traits, they inevitably raise adaptation/exaptation questions. Study of these suites may let us reconstruct the history that led to current interdependence of their component traits.

A second problem that is amenable to hormonal study concerns constraints, those "limits to improvement imposed by inherited form and function" that help explain why organisms "resist adaptive modification" (Gould 1986). A widely recognized form of genetic constraint is pleiotropy, which is often invoked to account for the expression of correlated traits, some of which are advantageous and others disadvantageous (see, e.g., Endler 1995; Sheldon and Whittingham 1997). Dawkins (1982), however, was not persuaded that pleiotropy was a "constraint on perfection." Over time, he argued, selection should favor modifier genes whose action would separate the multiple effects of pleiotropic genes so as to "reduce the harmful effect while enhancing the beneficial one" (Dawkins 1982, p. 35). Hormones may be one manifestation of pleiotropy: they are gene products (or are synthesized by gene products), and they have multiple effects. If the expression of an advantageous hormone-dependent trait requires the expression of a correlated trait such that the net effect on fitness is detrimental, then hormonal correlations can constrain adaptive evolution. But would we then expect selection to separate or unlink the multiple effects, following Dawkins's reasoning about pleiotropy? The circumstances under which separation would or would not occur have

received little study, and investigation of hormonal mechanisms should help resolve the issue.

A third problem, related to the second, concerns the focus (or unit, or level) of selection—that is, the question of when the focus is most profitably thought of as the gene, the trait, or the organism (Wilson 1997). Dawkins (1982) argued that once it is acknowledged that genes manifest themselves in effects extending beyond the individual organism (i.e., in the extended phenotype), then it becomes both logical and proper to consider the gene as the unit of selection. Genes that are selected are those that function best in their internal milieu (including other genes) and that cause their organism ("vehicle") to perform to best advantage in the external environment, including other organisms. Travis's (1989) emphasis, however, was on the organism as the unit of selection. Despite decades of debate and research, we still face the question of "whether evolution through selection has produced organisms with individually optimal trait values or whether it has produced an optimal organism as a consequence of genetically enforced compromises among critical traits" (Travis 1989, p. 292). Knowledge of the physiological (including hormonal), as well as the genetic, bases for correlated trait expression will be key to resolving this issue, because mechanisms controlling expression will determine when selection can act to bring traits under common control and when it can separate them.

The problems we have enumerated are beyond our ability to address adequately, particularly in a brief article. Rather, our goals are to highlight how a hormonal approach can help us understand the nature of some adaptations, exaptations, and constraints and to show how hormonal manipulations can be used to test adaptive hypotheses and identify constraints. In passing, we mention some of the methods now available for evolutionary studies of hormones.

We begin with a brief summary of relevant concepts from endocrinology and then consider examples of suites of interdependent hormonal traits. We divide these suites into two categories. In the first category are those whose component traits were connected historically, their expression thus controlled by a general, systemic cause, and, because of the connection, they were influenced as a group when selection acted on variation in a hormonal signal. In the second category are those whose components had independent histories that came to be correlated through selection on variation in their responsiveness to a systemic hormonal signal. In the first category, if only one of the component traits was the initial target of selection, others that were beneficial would be exaptations. Traits belonging to the second category, because they originated separately, would remain adaptations even if they later became more interdependent through selection for enhanced coordi-

nation. Although we regard these categories as representing a conceptually valid dichotomy, we recognize that their application to real cases will rarely if ever be simple. For example, in the same complex, evolution may have proceeded in separating the regulation of some correlated traits while integrating control of other traits that were once independent. Regardless, any effort to draw such distinctions will require an integrative program of research on hormonally correlated traits, one that will include study of the utility of the traits, their history, and their degree of interdependence of expression and response to selection.

Finally, we consider experimental studies, including our work on the dark-eyed junco (*Junco hyemalis*), in which hormones have been used to manipulate suites of phenotypic traits and to study their effects on fitness. The goals are to show how such studies have uncovered intraspecific variation on which selection might act and how they can contribute to the identification of constraints.

### Hormones and Traits

#### *About Hormones*

The summary that follows derives in part from information that can be found in any advanced text (e.g., Crews 1987; Becker et al. 1992; Norris 1997). It attempts to state just what is essential for appreciation of how natural selection might act on hormonal traits and notes parallels between pleiotropic genes and hormones and between epistasis and hormonal interactions.

Hormones are bioregulatory compounds that vary widely in chemical structure, although most are polypeptides, proteins, or steroids. A typical hormonal pathway in animals consists of a site of synthesis and secretion, often a gland, and several sites of action referred to as target tissues. Secretion can be stimulated by cues that originate in the external environment (e.g., by photoperiod or by exposure to a conspecific) or by cues from the internal environment (e.g., by another hormone). Targets can be local (autocrine), adjacent to (paracrine), or distant from (endocrine) the source and, in the last case, are typically reached via the circulatory system. At the targets are hormone receptors, which are inducible proteins and thus gene products; receptors may be located in the membrane of the target cell or inside the cell. Cells with receptors bind the hormone to form hormone-receptor complexes, and these complexes either alter cell function or initiate gene transcription. Cells that lack such receptors do not form these complexes. Peptide and protein hormones typically bind to receptors in cell membranes and act indirectly via second messengers, while steroid hormones typically bind to intracellular receptors to form steroid-

receptor complexes; these act as transcription factors binding in turn to hormone response elements within the promoter regions of genes and initiating transcription. But protein hormones sometimes initiate transcription, and steroids sometimes act directly (nongenomically) by altering membrane permeability (see, e.g., Orchinik and McEwen 1994).

After a hormone-receptor complex is formed, one response leads to another in what is called a cascade. For example, transcription of one gene may lead to a product that stimulates transcription of others. Applied to behavior, hormone-induced transcription might lead to synthesis of a neurotransmitter, permitting a neuron to transmit information to new locations and thus to elicit a previously unexpressed behavior. In general, the smaller the number of biochemical steps required to translate reception of the hormone into expression of a particular effect, the more direct that hormone's influence on the effect is said to be. Endocrinologists often refer to attributes or traits as hormone dependent or hormone mediated. The greater the number of links between secretion and effect, the less direct the causation and the more appropriate it is to say hormone mediated.

Not all tissues that respond to a hormone's presence do so at the same time, and hormones often produce their effects during critical periods in development. Some evidence suggests a model in which the organism is viewed as a collection of modules that rely on hormones for sequential or periodic activation. For example, at one of several targets, testosterone may require enzymatic conversion to estrogen or dihydrotestosterone before it can produce an effect. In the absence of the enzyme for conversion, and therefore of the active form of the hormone, there would be no response at that target and no ensuing cascade, despite the presence of the circulating form (e.g., birds: Harding et al. 1988; 5 $\alpha$ -reductase deficiency in humans: Norris 1997, p. 404).

Much remains to be learned about mechanisms regulating which tissues respond to hormones and when they do so. All of the following will prove important: the coordination of receptors with respect to their synthesis (i.e., locations, timing, and abundance); the existence of receptors that differ in kind but respond to the same hormone (as in the multiple receptors for estrogen; Rissman et al. 1997); and the presence of transcription factors that cause different cells to respond to the same hormone but transcribe different genes. But the fundamental notion of a single signal that produces multiple effects by its impact on an array of targets provides a good model for thinking about the action of hormones. There is obvious analogy with the way evolutionary biologists think about pleiotropic genes.

Physiological epistasis (Cheverud and Routman 1995)

also has a parallel in interactions among hormones. One form of endocrine interaction involves cases in which hormones act in linear sequences, the secretion of one fostering or suppressing the secretion of another. Hormones that act upstream (earlier in a sequence) have the potential to influence more traits than those acting downstream, because they produce not only direct effects but also indirect effects by influencing other hormones. In addition to sequential actions, a hormone can interact with another hormone at a target to permit, inhibit, or synergistically enhance expression of a single trait. For example, both estradiol and progesterone are necessary for the induction of maternal behavior in rats (Rosenblatt 1992), and both dihydrotestosterone and testosterone are required for normal penile development in humans (Norris 1997, p. 404).

Endocrinology comes alive for evolutionary biologists when they begin to consider sources of variation in hormonally mediated traits. Individuals that differ in phenotype, or species that have diverged into descendent lineages, might owe their differences to variation in one or all of the following: sensitivity to stimuli promoting or suppressing secretion of the hormone (with potential systemic effects), transport mechanisms and half-life of the hormone in the circulation (also systemic), and the localization, capacity, or affinity of receptors (i.e., responsiveness to the hormone). Equally important for phenotypic variation is variation in interactions among hormones and in timing of hormonal action during development. All of these sources of variation are subject to gene-based differences (Crawley and Praylor 1997; Nelson 1997; Rissman et al. 1997), as well as to environmental influences, including maternal effects (e.g., Runfeldt and Wingfield 1985; Schwabl 1993, 1996a, 1996b; Clark and Galef 1995; Schwabl et al. 1997).

#### *Studying Hormonal Effects*

To demonstrate that the expression of a trait is mediated by a particular hormone, one has traditionally needed to show that expression of the focal trait correlates with the level of the hormone or the sensitivity of the target tissues, is suppressed when the hormone is removed experimentally at its source or is blocked from acting on its receptors, and is restored when the hormone is replaced. The techniques for such demonstrations have become increasingly sophisticated, but the basic rationale has remained the same. As examples of changes in techniques, competitive binding assays (radioimmunoassays, or RIAs) have replaced bioassays to measure hormone concentrations. Agonists and antagonists that bind hormones or receptors in ways that prevent them from interacting with one another are used to supplement findings from surgical removals

of glands. Implants of porous tubing, pellets, and osmotic pumps permit long-term, standardized systemic application of hormones as well as localized delivery to particular target tissues; these methods replace the traditional need to apply hormones with periodic injections. Most recently, in an explosion of studies, molecular “knockout” techniques are used to block, permanently or temporarily, the transcription of genes that code for hormones or receptors (Crawley and Paylor 1997; Lederhendler 1997; Nelson 1997). At present, evolutionary biologists have applied some of these techniques to the study of hormonally based phenotypic effects in nature, but future expansion of these applications is almost certain.

Our interest is testosterone in birds, and we therefore use that hormone to illustrate the foregoing generalizations (see Balthazart 1983 for a still excellent review of testosterone’s numerous effects in birds). Autoradiography (which localizes receptors by applying radiolabeled hormone to tissue sections) and immunocytochemistry (ICC, which is based on immune reactions to receptor proteins) have detected receptors for testosterone at numerous sites both in the brain (among them sites associated with aggression, vocal behavior, and sexual behavior; e.g., Arnold et al. 1976; Balthazart and Ball 1993) and in the periphery (including skin, syrinx or vocal organ, uropygial or preen gland, and gonads; e.g., Lieberburg and Nottebohm 1979; Luine et al. 1980; Lake 1981; Scanes 1986; Shanbhag and Sharp 1996).

#### *Hormones and Traits*

Endocrinologists tend to see hormones as the regulators of physiology and strive to learn how they influence such processes as reproduction, digestion, osmoregulation, growth, and development. Evolutionary biologists who work with hormones are more likely to ask what maintains variation in fitness traits that are mediated by hormones and, in more concrete terms, what is changed behaviorally, physiologically, and anatomically, as well as genetically, when selection favors or acts against a hormonally mediated trait.

In considering hormone-mediated traits and asking whether selection acts on traits or organisms, we recognize that a key issue is the question of what constitutes a trait. As an example of this problem, consider the ways in which testosterone might affect vocalizations of the grey partridge (*Perdix perdix*). In this species, female mate choice is based at least in part on the male’s vocal attributes (Béani and Dessi-Fulgheri 1995). Males treated with testosterone produce more attractive calls, which are of longer duration than those of untreated males and cover a broader range of frequencies (Fusani et al. 1994).

The differences in call duration and structure are likely

to be the result of testosterone's effects on an array of androgen-sensitive targets that play a role in avian vocalizations. These targets may include regions of the brain that transmit temporally patterned impulses to the syrinx, muscles of the syrinx that influence a call's pitch (Béani et al. 1995; cf. Groothuis and Meeuwissen 1992), respiratory muscles controlling air pressure during exhalation (Béani et al. 1995), and muscles influencing the extent to which a calling male stretches his neck (which affects the resonant properties of the call; Fusani et al. 1994, 1997). Thus, if we ask how sexual selection might act on testosterone in male grey partridge, it seems likely that it would detect differences in the net effect of the hormone on a variety of features that combine to influence vocal behavior and male attractiveness. These differences might reflect variation in the level of testosterone in the system and/or the responsiveness of one or more androgen-sensitive targets at any of the above locations to any particular level of the hormone. If each component of the system is free to evolve independently, then more attractive calls may be achieved by varying the targets in any one of many possible combinations (e.g., lowering a call's pitch while enhancing its resonance). Each component of the call that varies independently would be a separate trait. But if production of a more attractive vocalization depends on a coordinated and integrated collective response of the components, then the call and not its components would be the trait under selection. We next consider how to assess degree of independence or interdependence of hormonally mediated attributes and how to relate an assessment to the concepts of adaptation and exaptation.

#### Applying the Adaptation/Exaptation Distinction to Hormonal Traits

When several advantageous traits form a complex because they are consequences of an integrating biosignal such as a hormone, we may ask whether it is possible to determine which members of the complex are adaptations and which are exaptations (Lande and Arnold 1983; West-Eberhard 1992*a*). If only traits originally and directly acted on by selection qualify as adaptations, as most modern definitions of adaptation require, then correlated but not directly selected hormone-mediated traits, however beneficial, should be classified as exaptations. Accurate assignment of traits to the appropriate category thus requires knowledge of their historic ability to respond independently to selection (West-Eberhard 1992*a*). At one extreme are correlations that are evolutionarily inseparable in the sense that selection's acting on a single trait leads inevitably to changes in the others. In such a case, only the trait that was the original target of selection would be an adaptation. At the other extreme are correlations that can readily be

broken, allowing selection independently to add or delete one or more traits of a complex. In this case, a target of selection could become an adaptation without engendering exaptations; all independently added elements of the complex could be adaptations.

Knowledge of the mechanisms underlying trait expression and how they produce variation is a key to distinguishing these extremes. To oversimplify, studies of particular cases would need to determine whether traits and the correlations among them tend to vary as the result of variation in systemic hormones or variation in responsiveness of target tissues to invariant levels of systemic hormones. Consider a complex of traits in which at least one component is an adaptation that evolved as a result of the advantages it provided in a particular environment. The question is whether the traits that are correlated with it are adaptations or exaptations. If the component that is the adaptation resulted from selection on variation in the levels of a systemic hormone, then, because the hormone would have reached all tissues, other traits correlated in expression would have made an unselected evolutionary response and, if beneficial, become exaptations. But if the selection that produced the adaptation acted on variation in the responsiveness of one or more target tissues to a hormone (e.g., variation in receptor density, in enzymatic conversion to an active form at the targets, or in presence/absence of a hormone-response element in the promoter regions of genes) and not on variation in the hormone's level, then evolution of more than one adaptation could have proceeded independently, and correlated traits could all be adaptations.

Working with a currently existing system, an investigator of this problem of correlated traits could accumulate various kinds of information as part of a research program. Acquiring knowledge of the complexity of the biochemical pathways involved in the expression of the traits would be important. Pathways with more steps between the stimulus for and the expression of the trait would be subject to influences at each step and therefore more likely to be independently modifiable by change at one of the steps. Application of systemic hormones in varying doses and at varying times in development, followed by measurement of impacts on trait development, would provide an assessment of trait interdependence. Estimates of heritability and of environmental effects on trait expression, as well as artificial selection on one or another member of a complex, would reveal the potential for correlated or independent response in the other traits (e.g., Sefton and Siegel 1975; Cunningham and Siegel 1978; Garland 1988; Siegel and Dunnington 1990; Bakker 1994; Zera and Zhang 1995; Van Tienderen and Van Hinsberg 1996; Swallow et al. 1998).

To probe conditions at the time a trait or traits evolved,

however, other approaches are required (Sheldon and Whittingham 1997). It is essential to examine phylogenies to determine whether, within a clade, traits arose as a complex in one member of the clade and were passed along as such or, alternatively, whether traits expressed as a complex in one member appear or disappear independently among relatives, apparently shifting one at a time from hormone dependence to hormone independence, or vice versa. Also important would be a comparison of environments, historical or modern, to search for evidence of convergence in the form of correspondence between environments and the presence of some or all of the hormone-mediated traits that sometimes appear as complexes. This approach would serve to relate individual traits or complexes to particular selection pressures.

To summarize the four preceding paragraphs, tracing the evolution of the components of a complex of hormonally mediated traits for the purpose of distinguishing adaptation from exaptation requires knowledge of mechanism and history. The obvious difficulty of this task explains why few adaptive-exaptive analyses of hormone-dependent complexes have been attempted to date (but see Fahrbach 1997). It is possible, however, to illustrate the first necessary step—that is, seeking knowledge of the relevant endocrinological mechanism. We consider two cases, one in which traits are likely to have evolved as elements of an adaptive-exaptive complex because selection acted on variation in a systemic hormone, and another in which adaptations seem to have arisen because of enhancement of responsiveness by independent hormonal targets.

*Traits That Are Tightly Associated: Hormones with Systemic Effects*

Spotted hyenas (*Crocuta crocuta*) present a classic problem in distinguishing between adaptation and exaptation. These animals have long fascinated biologists because females develop an erectile clitoris closely resembling a penis (Gould 1981; East et al. 1993; East and Hofer 1997; Frank 1997a, 1997b). In addition, female hyenas are larger than males and more aggressive. One of several proposed adaptive explanations for the evolution of masculinized female genitalia is that the penile clitoris plays a signaling role in a socially important display known as the greeting ceremony (Wickler 1966; Eibl-Eibesfeldt 1970; Kruuk 1972).

In their classic article introducing the concept of exaptation, Gould and Vrba (1982) argued that the penile clitoris is not an adaptation because it was not originally selected for its own sake. More likely, they argued, it was a correlated developmental consequence of selection for hormonally based female aggressiveness; that is, it had no original function. As an unselected but beneficial trait, its

role in the greeting ceremony would be an exaptation. Current students of hyenas (East et al. 1993; Frank 1997a) seem to agree that the female penile clitoris is probably an unselected side effect of selection for female aggressiveness, although they disagree on how aggressiveness benefits females (East et al. 1993; East and Hofer 1997; Frank 1997a, 1997b).

The argument for exaptation is based on research that has shown that female hyenas are masculinized largely as the result of a few simple regulatory changes from the ancestral condition, changes that together increase exposure of developing embryos to maternally derived testosterone (Yalcinkaya et al. 1993). In the typical placental mammal, the enzyme aromatase converts a maternal androgen (androstenedione) to estrogen before the androgen reaches the developing embryo. Levels of aromatase are low in the female hyena. Rather, an enzyme in the placenta (17- $\beta$  hydroxysteroid dehydrogenase) converts androstenedione to testosterone with the result that developing embryos, including female embryos, are exposed to unusually high levels of this hormone (Licht et al. 1998). The numerous results include masculinization in females of adult body size, genital form, aggressive behavior when young and adult, and greeting behavior. Based on these observations, it would seem that if only one of these traits was the historical focus of natural selection, the others would have been carried along in the wake of selection, becoming exaptations (Gould and Vrba 1982).

Two important questions remain, however, both raised by West-Eberhard (1992a). First, if it is true that the penile clitoris arose as an unselected side effect, how much post-origin modification under direct selection would be required before the structure qualified in its own right as a later-arising adaptation? Second, if the penile clitoris was part of a complex of traits that arose simultaneously as the result of a regulatory mutation, should the complex itself, and not one or more of its components, be considered the adaptation?

Regarding the first question, a number of observations indicate that the masculinization of the female hyena, particularly of her clitoris, involves more than early exposure to enhanced testosterone (Frank 1997a). For example, chemical treatment of pregnant spotted hyenas with anti-androgens produces a shorter, thicker clitoris in their offspring, but the modified structure is nevertheless large and erectile and quite unlike the clitoris of a typical mammal (Drea et al. 1998). This finding implies that full development of the peniform clitoris involves not simply early androgen exposure but also the action of steroid-independent mechanisms in the target tissue (Foster et al. 1996; Drea et al. 1998; Glickman et al. 1998; Licht et al. 1998). If so, and if the genes responsible for elaboration of the clitoris came to be favored after the penile form originated,

then Gould and Vrba's (1982) conclusion that the penile clitoris of the female spotted hyena is simply an unselected indirect effect of selection for other masculine traits might require reexamination (West-Eberhard 1992a).

Regarding the second question about the conceptual status of a complex and its elements (one adaptation or several?) a key consideration may be historical: when the complex arose, were the traits evolving "for the first time," or were they co-opted as a group and expressed in a new context. That is, knowledge of the mechanisms underlying the masculinization of the female hyena makes it seem possible that at one time, when the regulatory mutation in maternal aromatase arose, natural selection was presented with two classes of female spotted hyenas, some masculinized and some not. If a preexisting suite of traits normally expressed by males came to be jointly and simultaneously expressed by some females owing to an increase in embryonic exposure to testosterone, components of the suite would have been jointly and simultaneously acted on by selection. In this hypothetical situation it could be argued that the entire complex that originally evolved in males, and not its individual units, is the adaptation. This is the view of West-Eberhard (1992a, p. 16; emphasis added), who wrote, "new adaptations may sometimes originate as coadapted character sets, whose expression has been shifted between sexes or life stages (via heterochrony) and then modified in the new context (see West-Eberhard 1989)." Similarly, Arnold (1996) has discussed how genes and hormones can "capture" developmental processes by gaining control of cascades of developmental events. As an example of a gene's capturing a cascade, he cites the role of the SRY gene in the differentiation of the mammalian testes; and as an example of a hormone's capturing a cascade, he cites the role of gonadal steroids in the sexual differentiation of the mammalian brain (Arnold 1996).

#### *The Hormone Complex as an Adaptation*

Supporting evidence that altered regulation of systemic hormones can play a role in substituting one whole complex of traits for another comes from studies of hormonal regulation of the development of alternative phenotypes (see Moore 1991 for review). We list some striking examples: manipulation of thyroid hormone in the diet of spadefoot toads (*Scaphiopus multiplicatus*) alters the ratio of tadpoles that develop as omnivores to those that develop as carnivores. These naturally occurring phenotypes differ in diet, tendency toward cannibalism, digestive system, and rate of development (Pfennig 1990, 1992a, 1992b; see also Denver 1997). Manipulation of testosterone in hatchling tree lizards (*Urosaurus ornatus*) affects the proportions of males that develop as orange- or blue-throated morphs, and these naturally occurring phenotypes differ in terri-

toriality, aggressiveness, and body size (Hews et al. 1994; Hews and Moore 1995, 1996). In crickets (*Gryllus* spp.), early manipulation of juvenile hormone (JH) affects the ratio of two forms, one that delays breeding and disperses and another that matures early and does not disperse (Zera et al. 1998). As a final example, gonadotropin-releasing hormone (GnRH) is thought to influence development as an early-maturing or an alternative late-maturing form of the midshipman (*Porichthys notatus*), a marine fish (Grober et al. 1994). Further research will almost certainly reveal more cases in which a developmental change in production of a systemic hormone accounts for substitution of one set of traits for another (Moore 1991). Whether similar processes have played an important role in the origin of complex adaptations (West-Eberhard 1992a, 1992b) remains to be seen, but the conceptual possibility has considerable appeal.

#### *Traits That Appear Separable: Target Responsiveness and Receptors*

In contrast to evolution as the result of alterations in the hormonal signal, endocrine-mediated phenotypes might evolve because of alterations in responsiveness of target tissues. The example that we present is taken from work by Insel and colleagues (Winslow et al. 1993; Young et al. 1997, 1998) and is based on the roles of the hormone oxytocin in rodent reproduction. Oxytocin (OT) is a peptide hormone produced in the brain (posterior pituitary); it can act both centrally (on other sites within the brain) and systemically (after entering the circulation). When the OT receptor (a G-protein located in the cell membrane) binds with OT, it activates second messengers that in turn modify cell function.

In rats, manipulations of OT, including its central administration and interference with its operation through antibodies or antagonists, have conclusively demonstrated the hormone's numerous roles in reproduction (summarized in Young et al. 1997). Among these are the promotion of sexual behavior, onset of labor, ejection of milk on giving birth, and maternal behavior. In mice, gene knockout techniques have been used to interfere with the synthesis of OT, revealing both similarities and differences between mice and rats. Just as in rats, OT-knockout mice cannot lactate. However, such mice do exhibit normal maternal behavior, which suggests that OT is essential to lactation in both rats and mice but is required for maternal behavior only in rats. The explanation for the difference appears to lie in evolved differences in responsiveness of brain nuclei to OT (as well as to influences that other hormones have on those nuclei; Young et al. 1997). Thus, traits that we would regard as members of a tightly linked complex if we examined only rats can nevertheless be ex-



pressed independently and therefore have the potential to evolve independently.

To pursue the question of how this separability might be achieved, consider an interesting difference in the behavior and the OT receptors of two vole species. The prairie vole (*Microtus ochrogaster*) exhibits male parental behavior and social monogamy (Getz et al. 1993), while its congener the montane vole (*Microtus montanus*), like most rodents (and other mammals), exhibits neither (Jannett 1982). Prairie voles also differ from montane voles in affiliative behavior (i.e., the formation of attachments to conspecifics). Social bonds with sexual partners, young, and parents are strong in prairie voles, and these attachments are expressed in time spent together and production of distress cries when separated. Montane voles do not form such bonds and exhibit no distress on separation. The distribution of OT receptors in the brain also differs between the species: autoradiography reveals three brain nuclei that bind OT in prairie voles but do not bind it in montane voles. In attempts to demonstrate a causal connection between these species differences in distribution of OT receptors and affiliative behavior, T. R. Insel's laboratory is striving to induce a pattern of expression of OT receptors in the brains of montane voles that is like the pattern found in prairie voles. They have isolated a regulatory sequence from the DNA of prairie voles that promotes transcription of the OT receptor and is located upstream from the sequence that codes for it. They have succeeded in introducing this regulatory sequence into a transgenic mouse and have shown that its presence in the mouse genome leads to development of brain receptors distributed in the same pattern that typifies the prairie vole's brain (Young et al. 1997). The next step will be to introduce this construct into the montane vole to learn whether it elicits both the receptor distribution and the social behavior characteristic of prairie voles. The ability to use transgenic approaches to alter a species' hormonal receptors and perhaps its behavior, all in a single generation, holds enormous promise for describing pathways that evolution might have followed in the production of species differences in complex endocrine traits.

The findings about the multiple effects of OT and its interspecific differences are relevant to the effort to distinguish between adaptations and exaptations. The role of OT in onset of labor and lactation in all rodents studied indicates that development of OT receptors in certain brain nuclei is apparently a trait shared throughout the rodent lineage. Receptors for OT at other locations are apparently quite derived, with associated effects limited to only a few members of the clade, as divergences in affiliative behavior demonstrate. Thus, the difference in hormonal mechanism and the capacity for independent expression and evolution of affiliative behavior indicate that

affiliative behavior is an adaptation in its own right. We suspect that further research on suites of hormonally mediated traits will disclose other examples comparable to those outlined here: at one extreme, adaptations produced by selection on variation in systemic hormones and accompanied by exaptations and, at the other extreme, sets of relatively independently derived adaptations resulting from selection on variation in the responsiveness of target tissues.

#### Hormonal Traits, Trade-Offs, and Constraints

Of course, not all traits mediated by hormones are advantageous. When some are advantageous and some disadvantageous, they can influence one another's evolution through trade-offs, which are "linkages between traits that constrain the simultaneous evolution of two or more traits" (Stearns 1992). Said another way, trade-offs are "fitness costs that occur when a beneficial change in one trait is linked to a detrimental change in another" (Stearns 1989). As we use the term, hormonal trade-offs are costs to potential fitness that occur when some but not all of the traits mediated by a hormone are beneficial. Its fitness would be greatest if the organism were free to express two hormone-mediated traits, A and B, each to its optimal level. But if altering expression of A to its optimum would require moving B from its optimum, then the evolution of A is constrained by its effect on B. To recognize trade-offs that act as insurmountable constraints, we apply the reasoning used earlier to distinguish adaptations from exaptations: we base the distinction on the level of interdependence of the mechanisms that regulate A and B. The more tightly associated a hormone is to the expression of two or more traits that conflict in their impact on fitness, the more likely the trade-off will represent a constraint. Three examples of hormonal trade-offs should illustrate.

Birds have traditionally interested evolutionary biologists because of their brightly colored plumage and other extravagant (usually male) traits, generally accepted as the results of sexual selection. One of the important ideas to account for the evolution of such traits was proposed by Hamilton and Zuk (1982), who suggested that females might prefer extravagance in male traits because only healthy males are capable of it. Several workers then noted that extravagance may represent a handicap because the hormonal physiology that underlies it may also enhance susceptibility to parasites or disease (Ligon et al. 1990; Zuk 1996; Zuk et al. 1990, 1995; Folstad and Karter 1992; Owens and Short 1995; Saino et al. 1995; Ros et al. 1997; but see Weatherhead et al. 1993; Siva-Jothy 1995). The extravagance handicap is assumed for the advantages it confers in mate choice; the cost that the handicap imposes also constrains the evolution of further extravagance. Sim-

ilar connections among hormones, sexual selection, and parasites have recently been made in other groups of organisms (e.g., Salvador et al. 1996; Klein et al. 1997). While the idea of a hormonal handicap has its critics (see, e.g., Owens and Short 1995; Hillgarth and Wingfield 1997 for review), it is compelling, and further work should reveal whether it is supported.

A different and particularly elegant example of a trade-off with an apparent endocrine basis comes from the work of Sinervo and colleagues (Sinervo and Huey 1990; Sinervo and Licht 1991a, 1991b; Sinervo et al. 1992), who documented the interaction between egg size and egg number (i.e., clutch size) in side-blotched lizards (*Uta stansburiana*). Follicle-stimulating hormone (FSH) promotes ovulation, and experimental application of FSH induced females to lay larger clutches. But these large FSH-induced clutches were composed of eggs that were smaller than normal, which gave rise to hatchlings that were smaller than normal, which did not survive as well as hatchlings from normal clutches. Reduced survivorship of the brood is the cost that constrains increase of brood size beyond current levels.

In the Mongolian gerbil (*Meriones unguiculatus*), we find a third example of a hormonal trade-off, one that is environmentally induced and nonheritable. Clark et al. (1992, 1997) reported that adult males vary in mating effort and parental effort as the result of naturally occurring variation in their hormonal environment when they were developing embryos. Male gerbils whose intrauterine position lies between two male siblings (2M males) are masculinized, as compared with males that develop between two female siblings (2F males). Behavioral studies showed that 2M males, when adult, are more successful than 2F males at impregnating multiple females (Clark et al. 1992). However, 2F males are more attentive to young than are 2M males, and females mated to 2F males have shorter interlitter intervals and produce larger second litters than females mated to 2M males (Clark et al. 1997). Several lines of evidence indicate that the differences between the two types of males are traceable to differences in their levels of testosterone when adult, which in turn are traceable to their maternal intrauterine environment when embryos. Because time allocated to courting females cannot be devoted to caring for young, gerbils trade gains in fitness resulting from increased mating effort (2M males) for gains resulting from increased parental effort (2F males), and vice versa (cf. Raouf et al. 1997).

The lines of research that generated these examples of hormonal trade-offs were not designed to identify the mechanisms that might account for the interdependence of the traits. In each case, whether change in one trait (e.g., number of eggs) constrains the evolution of the other (e.g., size of egg) depends on the separability of the traits by

natural selection. Here again, the answer to the question of separability requires investigation of mechanisms, studies of selection (artificial and natural) in action, application of comparative methods, and methods of historical ecology. In addition, it requires tests of the current utility of traits when they are expressed both in isolation and as parts of a complex.

### Relating Hormonal Traits to Fitness

Phenotypic manipulations allow investigators to assess the utility of traits by modifying them and comparing the fitness of modified individuals to that of typical, unmanipulated individuals (e.g., Mitchell-Olds and Shaw 1987; Grafen 1988; Schluter 1988; Sinervo and Basolo 1996). This approach to demonstrating current utility, which we have called phenotypic engineering (Ketterson and Nolan 1992; Ketterson et al. 1996), is an essential part of establishing whether particular traits are adaptations.

When single traits whose variation is normally distributed are experimentally altered, three classes of outcomes are expected: adaptive/exaptive, neutral, and paradoxical (see fitness profiles in Falconer 1989; Stearns 1992; Ketterson et al. 1996). In an adaptive/exaptive outcome, experimental individuals, in which the value of the salient trait is made to deviate in either direction from the norm, have lower fitness than controls, which represent the norm. A neutral outcome is one in which deviant individuals perform as well as controls, and a paradoxical outcome is one in which deviants have higher fitness than controls.

Classic examples of adaptive/exaptive outcomes of manipulations of a single trait include experimental darkening of the normally red epaulets in red-winged blackbirds (*Agelaius phoeniceus*; Peek 1972; Smith 1972) and experimental shortening of the long corolla spurs in *Plantathea* orchids (Nilsson 1988). In both instances, deviants lost fitness; that is, red epaulets and normally long corolla spurs have current utility and may be adaptations.

Especially intriguing are results in which the experimentally created phenotype outperforms the typical, natural form to produce the paradoxical outcome. A classic study of this type is Andersson's (1982) tail elongation experiment on long-tailed widowbirds (*Euplectes progne*), in which males with experimentally elongated tails attracted more mates than males with tails of normal length (for similar results, see Burley 1986; Saino et al. 1997; Burley and Symanski 1998). Paradoxical results lead one to ask why the apparently advantageous attribute has not evolved, and two classes of explanatory answer are common. In the first class, counterselective pressures, not constraints, may block evolution of the apparently advantageous trait. For example, if we knew all the fitness consequences of long tails, we might find that they attract

predators as well as mates or impair maneuverability in flight (Møller 1994). In the second class, which involves constraints, the focal trait may be correlated in expression with other, potentially disadvantageous traits, and it is the action of selection on these related traits that sets a limit on the evolution of the focal trait. For example, to learn whether elongation of the tail of the widowbird is constrained in nature in this way, a next experimental step would be to induce growth of the tail and to identify any accompanying phenotypic effects, then to determine whether the collective (net) impact of all alterations on fitness is positive or negative. If longer tails were accompanied, for example, by longer bills and the net effect on fitness were negative, then we might conclude that tail growth is constrained.

Manipulations that alter suites of traits, as opposed to the single-trait experiments described earlier, offer new opportunities to investigators. Such manipulations may produce some changes that are advantageous, and others that are disadvantageous, which permits the identification of constraints by revealing how the fitness consequences of one trait might confine evolution of another. Manipulations of the external environment can be used in this way, as two outstanding studies in this issue reveal. By means of heat shock in *Drosophila*, Feder (1999, in this issue) has produced phenocopies that are normally the result of mutations; and by varying light quality, Dudley and Schmitt (1996; Schmitt 1999, in this issue) have induced the shade avoidance response in plants (see also Van Tienderen and Van Hinsberg 1996). Both environmental and hormonal manipulations are especially interesting because they elicit phenotypes that the organism is capable of expressing but for some reason does not. The objective is to identify the reason.

The past 10 yr have seen a number of studies in which investigators have altered suites of traits by manipulating systemic hormones and quantifying phenotypic effects and attempting to relate these to components of fitness. In mountain spiny lizards *Sceloporus jarrovi*, experimentally enhanced testosterone affected appetite, activity rhythms, and metabolic rate, and it also depressed survivorship (Marler and Moore 1988, 1989, 1991; Marler et al. 1996). In the side-blotched lizard *Uta stansburiana*, experimentally enhanced FSH influenced egg size, egg number, and hatchling size, and it also reduced hatchling survivorship (see "Hormonal Traits, Trade-Offs, and Constraints"; Sinervo and Huey 1990; Sinervo and Licht 1991a, 1991b; Sinervo et al. 1992). In brook trout *Oncorhynchus mykiss*, experimentally enhanced growth hormone increased metabolic rate, feeding behavior, and aggression (Johnsson et al. 1996; Jönssen et al. 1996). The hormone also caused young trout to behave in ways that would enhance risk of predation (Jönssen et al. 1996). This approach has also

been employed by plant evolutionary biologists. For example, in *Plantago lanceolata*, application of the hormone gibberellic acid induced plants to produce rosettes with fewer but longer and more erect leaves, whereas application of an inhibitor of gibberellin synthesis had the opposite effects (Van Tienderen and Van Hinsberg 1996).

Manipulations have also shown how hormones can influence allocation of effort to alternative morphologies in ways that emphasize reproduction at the expense of survival and vice versa. For example, JH influences relative growth of horns as opposed to eyes in dung beetles (Emlen and Nijhout 1999). In *Gryllus* crickets, JH influences relative growth of ovarian tissue as opposed to flight musculature (Zera et al. 1997, 1998). Similarly, testosterone influences allocation of effort to competing behavioral strategies in male birds, as will be shown in the next section.

We anticipate that investigators in the future will begin to devote increasing effort to modifying hormonally mediated traits, both in suites and in isolation (e.g., by applying a hormone both systemically and at particular targets). By measuring the utility (effects on fitness) of a hormone-mediated trait when expressed as a member of a suite and when expressed alone, it should be possible to establish a trait's potential and its realized utility. By computing the difference between these two measures, it should be possible to express the magnitude of any constraint in units of fitness.

#### Testosterone, Mating Effort, and Parental Effort in Birds

In this final section, we summarize our own research efforts involving systemic application of testosterone to a species of bird, the dark-eyed junco (*Junco hyemalis*: Emberizidae). Among birds in general, natural variation in the hormone testosterone has been related to variation in mating system. Elevation of plasma testosterone throughout the breeding season is characteristic of males in polygynous species, whereas a single peak of testosterone early in the breeding season characterizes socially monogamous males (Wingfield et al. 1987, 1990; Beletsky et al. 1995). Males of polygynous and monogamous species also differ in the way that they allocate effort between obtaining mates and caring for young, and the difference in their testosterone profiles may account for this difference in allocation, which is widespread among bird species. Thus, the consequence of experimentally elevating testosterone has been to shift male reproductive effort away from care of offspring toward other forms of reproductive effort, such as singing and sexual and aggressive behavior (e.g., Beletsky et al. 1995; see citations in table 1). In some cases, elevation of testosterone has also compromised male sur-

**Table 1:** Avian studies in which testosterone has enhanced mating effort, suppressed parental effort, and produced physiological consequences expected to shorten life span

Species	Source
<b>Mating effort:</b>	
Grey partridge <i>Perdix perdix</i>	Fusani et al. 1997
Sharp-tailed grouse <i>Tympanuchus phasianellus</i>	Trobec and Oring 1972
Red grouse <i>Lagopus lagopus</i>	Watson and Parr 1981
Red jungle fowl <i>Gallus gallus</i>	Zuk et al. 1995
Black grouse <i>Tetrao tetrix</i>	Alatalo et al. 1996
Barn swallow <i>Hirundo rustica</i>	Saino and Møller 1995; Saino et al. 1995
White-crowned sparrow <i>Zonotrichia leucophrys</i>	Wingfield 1984
Song sparrow <i>Melospiza melodia</i>	Wingfield 1984
Satin bowerbird <i>Ptilonorhynchus violaceus</i>	Collis and Borgia 1992
Dark-eyed junco <i>Junco hyemalis</i>	Chandler et al. 1994; Enstrom et al. 1997
<b>Parental effort:</b>	
Pied flycatcher <i>Ficedula hypoleuca</i>	Silverin 1980
House sparrow <i>Passer domesticus</i>	Hegner and Wingfield 1987
Reed warbler <i>Acrocephalus scirpaceus</i>	Dittami et al. 1991
Spotted sandpiper <i>Actitis macularia</i>	Oring et al. 1989
Dark-eyed junco <i>Junco hyemalis</i>	Ketterson et al. 1992, 1996
Barn swallow <i>Hirundo rustica</i>	Saino and Møller 1995
Yellow-headed blackbird <i>Xanthocephalus xanthocephalus</i>	Beletsky et al. 1995
Red-winged blackbird <i>Agelaius phoeniceus</i>	Beletsky et al. 1995
<b>Life span:</b>	
Red jungle fowl <i>Gallus gallus</i>	Ligon et al. 1990; Zuk 1990; Zuk et al. 1990
Black-headed gull <i>Larus ridibundus</i>	Ros et al. 1997
Barn swallow <i>Hirundo rustica</i>	Saino et al. 1995
Brown-headed cowbird <i>Molothrus ater</i>	Duffy 1989
White-crowned sparrow <i>Zonotrichia leucophrys</i>	Hillgarth and Wingfield 1997
Dark-eyed junco <i>Junco hyemalis</i>	Nolan et al. 1992

vival or produced phenotypic changes that would seem likely to have that effect (e.g., Hillgarth and Wingfield 1997; Hillgarth et al. 1997; see citations in table 1).

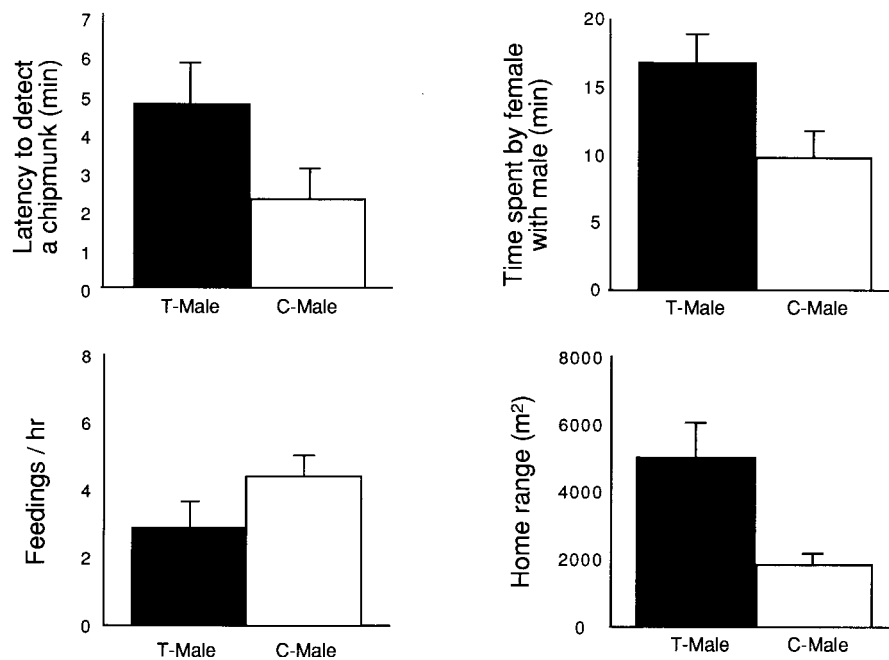
In applying testosterone to the junco, we anticipated that some testosterone-induced alterations might be advantageous but that others would be disadvantageous, and, because the manufactured phenotype deviated from the norm, we expected the net effect to be disadvantageous. That is, we expected an adaptive/exaptive outcome. We begin by presenting selected facts about the bird and describing the essence of our methods. We then summarize testosterone's effects on the junco's phenotype, pointing out how certain changes might alter fitness. We conclude the section by describing efforts to map testosterone's multiple effects onto measurements of fitness components made in the field.

#### *Dark-Eyed Junco Biology and Methods*

The junco is a socially monogamous songbird that is widely distributed in North America. Only females build nests, incubate eggs, and brood young; males establish and defend territories, attract mates, help defend eggs and

young against predators, and help feed young. Nest predation is heavy (reaching 80% in some years), and after such predation females typically remain with their mates and re-nest, sometimes repeatedly, until the end of the breeding season. Some females succeed in reproducing early enough to enable them to breed again that season. The result is that breeding becomes asynchronous among individual females, and throughout the season there are some females that are fertile (nest building, egg laying) and sought after by males. Many of the eggs laid (about 35%) are fertilized not by the laying females' social mates but by males from neighboring territories (extrapair fertilizations, or EPFs; Ketterson et al. 1997; Raouf et al. 1997). During winter, some individuals of the mountain-dwelling population that we study remain on or near their territories, and others make short-distance migrations to lower elevations (Ketterson et al. 1991a)

Since 1987, we have used silastic implants to raise plasma levels of testosterone (hereafter T) in free-living male juncos at Mountain Lake Biological Station in Virginia. Briefly, half the males caught in spring before or at about the time breeding has begun are randomly assigned to receive subcutaneous implants packed with T (experi-



**Figure 1:** Behavioral differences between testosterone-treated (T-male) and control (C-male) male dark-eyed juncos (mean  $\pm$  SE): time to detect a simulated nest predator placed near the nest (*upper left*; Cawthorn et al. 1998); female preference as measured by time spent in close association with either of two simultaneously presented males (*upper right*; Enstrom et al. 1997); rates of feeding nestlings (*lower left*; Ketterson et al. 1992); and home range size after egg laying completed (*lower right*; Chandler et al. 1994).

mental or T-males); the other half receive empty implants (control or C-males). All are individually marked and then immediately released where they were caught. In T-males, the implant causes circulating levels of testosterone to remain for the full season at the physiological peak level ordinarily exhibited by unmanipulated free-living males for a brief period in early spring (compare the season-long profiles of T-males with the natural profiles of polygynous species, described under “Testosterone, Mating Effort, and Parental Control Effort in Birds”; Ketterson and Nolan 1992; Chandler et al. 1997). The implants are generally removed in late summer.

#### *Phenotypic Effects of Treatment with Testosterone*

**Size of Home Range.** To assess the effect of T on use of space, Chandler et al. (1994, 1997) radio-tracked free-living male juncos at three stages of reproduction: when females were fertile (nest building, egg laying), were incubating eggs, and were caring for nestlings (assisted by males). When females were fertile, home ranges of treatment groups were similar in size, and T-males and C-males spent equal time closely accompanying (“guarding”) their mates (Chandler et al. 1997). But when females were incubating eggs or caring for nestlings, T-males’ home

ranges expanded and were three times larger than those of C-males (Chandler et al. 1994; fig. 1). Together, these findings predict that males of treatment groups would not differ in the extent to which they lose fitness as the result of fertilization of their females by neighboring males, but that T-males (when their mates are not fertile) would encounter more fertile female neighbors and would have greater success than C-males in gaining such EPFs.

**Response to Predators.** To assess whether the larger home ranges of T-males might result in decreased effectiveness in defending the nest, Cawthorn et al. (1998) placed a mounted chipmunk (*Tamias striatus*), the abundant and primary local nest predator, near nests and measured the responses of T- and C-males. Juncos are often successful in driving away live chipmunks, and when they detected the mount, they attacked it. However, T-males were less likely ( $P < .10$ ) than C-males to detect the mount (fig. 1; Cawthorn et al. 1998), which predicts that T-males should lose more eggs and young to nest predation.

**Mate Choice.** To assess the effect of T on male attractiveness, Enstrom et al. (1997) simultaneously presented dyads of T- and C-males to captive females and compared the amounts of time females spent with each category of male.

T-males courted (sang, behaved actively) more than C-males, and females spent more time with and were more likely to solicit copulation from T-males (fig. 1; Enstrom et al. 1997). Thus, behavior mediated by T enhanced male attractiveness, but we do not know whether, among free-living birds, the female preference for males with high T would manifest itself in choice of males as social mates, or as partners for EPFs, or both.

Plumage appearance and age also affect male attractiveness. The tails of juncos are partly white, and the white area is larger in older males than in yearlings. When Hill et al. (1999) manipulated this whiteness by replacing some dark feathers with white ones, females preferred males with whiter tails to males with normal tails (in which dark feathers had been replaced, but with other dark feathers). Since older males also have naturally higher levels of T (Ketterson and Nolan 1992), whiteness of tail and level of T normally covary with age. Hill et al. (1999) eliminated this correlation and put T level in opposition to tail whiteness by elevating T in males with normal tails and enlarging the white area in tails of males with normal T. Some females preferred males with whiter tails, and some preferred males with higher T (i.e., males that sang more frequently and displayed more actively). Thus, plumage and male behavior are both attractive, each in its own right, and the two can interact to affect female choice. We do not yet know whether level of T has an effect on tail whiteness. The answer should permit us to partition the independent and combined effects of testosterone on both behavior and appearance as they relate to attractiveness (cf. Møller et al. 1998).

*Sperm Reserves.* To ascertain whether treatment with T might affect a male's ability to inseminate females, Kast et al. (1998) collected ejaculates from free-living T- and C-males. Treatment had no detectable effect on sperm number when the season was considered as a whole, but in both groups of males sperm number was lowest when their social mates were fertile, and numbers were lower in T-males than C-males. These findings suggest that sperm numbers are depressed at the time when males would be expected to be copulating most frequently. They also suggest that T-males may copulate more often than C-males, which might make them more successful at siring offspring.

*Vocal Behavior.* To assess testosterone's effect on self-advertisement, we measured song. T-males sang more frequently than C-males (fig. 1; Ketterson et al. 1992; Chandler et al. 1994; Enstrom et al. 1997), which probably contributes to their greater ability to attract females (Enstrom et al. 1997) and may help to repel intruding male juncos seeking EPFs. We predicted that more frequent

singing might lead to greater success at siring offspring and fewer losses of paternity as the result of EPFs of their social mates.

However, unlike males implanted with T early in the breeding season, captive yearling males treated with T in late winter (i.e., before their first breeding season) sang less often and had smaller song repertoires than C-males (Titus et al. 1997). This suggests that untreated males in nature might be selected against if their T levels rose abnormally early (e.g., in late winter). Stated more broadly, it is possible that selection for elevated T during breeding might lead to a correlated elevation at a season or stage of life when high T was disadvantageous. If so, this might constrain T level during breeding.

*Parental Behavior.* To assess testosterone's effect on parental expenditure, we observed behavior at the nest. T-males fed their nestlings less often than C-males (Ketterson et al. 1992). However, females mated to T-males fed young more frequently than females mated to C-males, apparently compensating for the reduced male help. The somewhat surprising result was that the total number of parental feeding visits to the nest did not differ according to treatment of the male, nor did the body mass (condition) of nestlings (fig. 1; Ketterson et al. 1992). Nevertheless, we anticipated that the social mates of T-males might rear fewer fledglings than the mates of C-males, and we predicted that these "overworked" females might be more likely to switch mates between breeding attempts in the same or subsequent years.

*Response to Stressors.* To assess whether elevated testosterone might affect male health, we measured plasma levels of the stress hormone corticosterone and found that they were higher in T-males than in C-males (Ketterson et al. 1991b; Klukowski et al. 1997; Schoech et al. 1999). Free-living T-males also responded more strongly than C-males to the stress of being captured and handled: corticosterone rose in males of both treatment groups during the first few minutes following capture, but the rise was steeper in T-males (Schoech et al. 1999). These results—tonically higher circulating corticosterone and greater sensitivity to a stressful experience—are consistent with the possibility that T induces a physiological state of stress. Such a state is often linked to suppression of the immune system and increased susceptibility to disease and would predict lower survivorship of T-males (Hillgarth and Wingfield 1997).

*Molt.* To assess effects of exogenous testosterone when present in the system "out of season," we examined molt in juncos whose implants were not removed in late summer (Nolan et al. 1992). Juncos normally undergo a complete molt (i.e., all feathers are replaced) in the late summer

to early fall, after breeding. In T-males whose implants were removed no later than October, molt, though delayed in onset, was nevertheless completed. But T-males that retained their implants into the winter either did not molt at all or underwent an incomplete molt (Nolan et al. 1992). Feathers that did not molt were faded in comparison with freshly grown feathers and because of abrasion were much reduced in substance. Presumably they provided less effective insulation, may impair aerodynamic competence, and may (because of their faded color) make males less attractive.

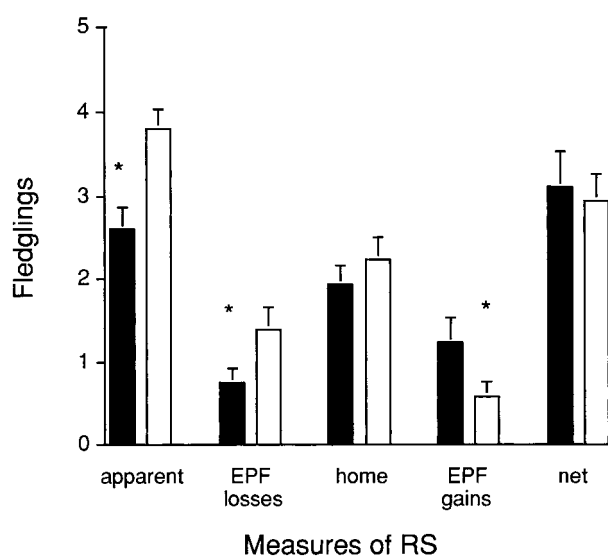
**Body Fat.** Finally, to assess testosterone's effect on male condition, we measured body mass and usually inspected subcutaneous fat stores during the breeding season and found no differences between T- and C-males (Ketterson et al. 1991b). However, in 1 yr when implants were administered prematurely (6 wk before the normal seasonal peak in circulating T), the effect was to suppress body mass and reduce fat deposits (Ketterson et al. 1991b). As was the case with vocal behavior (described earlier), this result suggests that if selection for elevated T during breeding is correlated with its elevation earlier in the year when high T might be disadvantageous, T level during breeding could be constrained.

In sum, we have found that altering the male junco's typical seasonal pattern of plasma testosterone produces multiple phenotypic effects. Was the net result adaptive/exaptive, paradoxical, or neutral? To answer this question we attempted to relate these experimentally induced effects to fitness.

#### *Fitness Consequences of Treatment with Testosterone*

**Reproductive Success (RS).** "Apparent RS" refers to the number of young produced in the nests of the social mate of a male, without regard to the genetic relatedness of the young to that male (i.e., ignoring possible EPFs). By this measure, T-males produced significantly fewer fledglings than do C-males, on both a per brood (Ketterson et al. 1996) and per season basis (Raouf et al. 1997; fig. 2). However, because males frequently fertilize the eggs of females mated to neighboring males and also suffer fitness losses when the eggs of their social mates are fertilized by neighbors (Ketterson et al. 1996, 1997; Raouf et al. 1997), apparent RS is not an accurate measure of true RS. Use of multilocus minisatellite DNA fingerprinting to assess season-long genetic RS revealed important differences between T- and C-male juncos (Raouf et al. 1997; fig. 2). Although apparent RS was greater in C-males, C-males also suffered greater loss of paternity to EPFs.

The net result was that when we consider only the true (genetic) production of young in the nests of their social



**Figure 2:** Five measures of reproductive success (RS; mean  $\pm$  SE) in testosterone-treated (T-male; solid bars,  $n=58$ ) and control (C-male; open bars,  $n=67$ ) male dark-eyed juncos: apparent success, extrapair fertilization (EPF) losses, home success (apparent success minus EPF losses), EPF gains, and net success (home success plus EPF gains; from Raouf et al. 1997). Asterisk indicates  $P < .05$  after correcting for multiple comparisons.

mates (apparent success minus losses to EPFs in the "home" nests), genetic RS did not differ detectably between C- and T-males (fig. 2). Further, EPF gains (i.e., young sired in the nests of females mated to other males) were greater for T-males than for C-males (fig. 2). The overall effect was that net genetic RS (apparent RS minus EPF losses plus EPF gains) did not differ according to male treatment (fig. 2). Although treatment did not affect net success, C-males were more likely to achieve genetic RS through parental effort (caring for young in the nests of their social mates), while T-males were more likely to achieve it by mating effort (limiting "home" losses resulting from EPFs of their social mates and gaining more EPFs for themselves at the expense of their neighbors; Raouf et al. 1997; fig. 2).

**Remating.** Females mated to T-males have lower reproductive success than females mated to C-males, which led us to expect that females mated to T-males might desert their mates and choose new ones when they next bred. However, females were as faithful to T- as to C-males, both when they renested within a season and when they selected mates in subsequent breeding seasons (Ketterson and Nolan 1992; Ketterson et al. 1996).

**Adult Survival.** Survival from year to year (measured as

return rate in the year following treatment) of T- and C-males depended on whether and when the implant was removed in the year of treatment. When implants were removed in late summer, the usual procedure, T-males were at least as likely as C-males to return and breed the next year (58% T vs. 48% C; Nolan et al. 1992; Ketterson et al. 1996). Removal as late as October also did not influence survival (50% T vs. 50% C; Nolan et al. 1992). However, when implants were not removed, T-males were significantly less likely than C-males to return (20% T vs. 43% C; Nolan et al. 1992). We conclude that suppression of molt or some other consequence of elevated T in autumn resulted in higher mortality of T-males.

*Interpretation.* We had anticipated that the natural pattern of plasma testosterone would produce a set of traits that was more beneficial than our engineered set, but so far we have not found that experimental prolongation of elevated T produces significant reductions in reproductive success or life span (except when we did not remove implants). Therefore, it is not yet clear why the natural range of hormonal phenotypes in the male junco does not include more individuals with elevated plasma testosterone like that of the experimental phenotypes. We continue to expect that the natural pattern of T confers greater fitness (the adaptive/exaptive result) and now consider possible explanations for not having found that result to date.

Perhaps T-males are at a disadvantage (and thus males with naturally elevated levels of T might also be at a disadvantage) with respect to some component(s) of fitness that we have not yet measured or that we have not measured with sufficient statistical power or at the "right" time. The study is ongoing, and we are considering new fitness components, such as the effect of T on immunocompetence and susceptibility to disease (J. M. Casto et al., unpublished manuscript; S. P. Hudman et al., unpublished manuscript) as well as survival of young after independence. We are also increasing the number of observations on components that we have already measured but with low power (e.g., rate of female desertion of T- and C-males). As the study proceeds, we may encounter breeding seasons in which the weather will be worse than we have encountered so far. When food for nestlings is hard to find, females may be less able to compensate for reduced male help, with the result that net RS of T-males might be lower than that of C-males. Nest predation varies greatly from year to year (20%–80% of nesting attempts) and is positively correlated with the abundance of rodent nest predators (Ketterson et al. 1996). Years of high nest predation may be sufficiently common to make it generally advantageous for males to concentrate their reproductive effort on the success of the home nest rather than at-

tempting to sire young in neighboring nests whose probability of succumbing to predation is also high.

The remaining obvious possibility, as some of our findings suggest, is that elevation of T to our engineered breeding-season peak levels may be constrained by disadvantages not directly related to breeding: perhaps if endogenous T were maintained at peak levels throughout the breeding season, this could be accomplished only by elevating T at other times of year or stages of life, when it would impose prohibitive costs on males. The suppressive effects that late-winter (or early-spring) exposure to T has on song repertoires (Titus et al. 1997) and fat stores (Ketterson et al. 1991*b*) and that autumn exposure has on molt (Nolan et al. 1992) are clear signals that extreme temporal alteration of the natural T cycle could be disadvantageous (Ketterson et al. 1996). A still more remote possibility may be that genetic correlations between the sexes constrain the evolution of any radically altered T profile in males (Price and Burley 1993).

### Conclusion

Our primary objective in this article has been to indicate how information derived from studies of hormones might contribute to resolution of issues in the study of adaptation, chief among them how to distinguish adaptations from exaptations and whether and how hormones can constrain trait evolution. The multiple effects that hormones have on phenotypes and the roles of hormones in integrating organism-level responses to the environment argue strongly against the notion that evolution proceeds by altering organisms on a trait-by-independent-trait basis. Hormonal studies should enhance our understanding of limits to adaptation and also of the emergence of complex adaptations. A coordinated program of research on endocrine adaptations would include study of the responses of hormonal mechanisms and hormonally mediated traits to natural and artificial selection. The findings of such research should be combined with those of phylogenetic studies that examine the components of hormonally mediated complexes from the perspective of historical ecology. Comparisons of unrelated taxa to determine whether analogous traits developed along similar endocrinological pathways could reveal convergence, providing evidence of adaptation.

To illustrate the possibilities and the limitations of hormonal experiments whose perspective is evolutionary, we have summarized recent investigations that used hormones to manipulate correlated attributes of organisms. New genetic and hormonal techniques will permit alteration of individual elements of suites of traits by their targeted induction or elimination. These approaches will supplement more familiar methods such as the application



of systemic hormones. If experimental alteration of an element enhances fitness but the normal expression of the entire suite leads to less fitness, this would be concrete evidence of constraint. We would also understand the constraint's mechanistic basis, an exciting prospect indeed.

The question of whether the target of selection is the trait or the organism will continue to engender debate. Traits that can readily move under and out of hormonal control independently of one another (e.g., through simple changes in attributes of receptors) may each be a unit of selection. In contrast, the unit shifts toward the organism when selection has proceeded in the direction of correlating originally independent traits by bringing them increasingly under the control of coordinating signals (e.g., systemic hormones). And in cases in which complexes of traits emerge simultaneously as the result of mutations of regulatory genes controlling endocrine modules, it may be most useful to regard the module as the unit of selection. We conclude by agreeing with Reznick and Travis, who write that only by seeking a "confluence of evidence" can we hope to construct a "compelling empirical argument for adaptation" (1996, p. 260). Hormonal studies hold great promise of contributing to that evidence.

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#### Literature Cited

- Alatalo, R. V., J. Höglund, A. Lundberg, P. T. Rintamäki, and B. Silverin. 1996. Testosterone and male mating success on the black grouse leks. *Proceedings of the Royal Society of London B, Biological Sciences* 263: 1697–1702.
- Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature (London)* 299:818–820.
- Arnold, A. P. 1996. Genetically triggered sexual differentiation of brain and behavior. *Hormones and Behavior* 30:495–505.
- Arnold, A. P., F. Nottebohm, and D. W. Pfaff. 1976. Hormone concentrating cells in vocal control and other areas of the brain of the zebra finch (*Poephila guttata*). *Journal of Comparative Neurology* 165:487–511.
- Bakker, T. C. M. 1994. Evolution of aggressive behavior in threespine stickleback. Pages 345–380 in M. A. Bell and S. A. Foster, eds. *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford.
- Balthazart, J. 1983. Hormonal correlates of behavior. Pages 221–365 in D. S. Farner, J. R. King, and K. C. Parkes, eds. *Avian biology*. Vol. 7. Academic Press, New York.
- Balthazart, J., and G. F. Ball. 1993. Neurochemical differences in two steroid-sensitive areas mediating reproductive behaviors. Pages 133–161 in R. Giles, ed. *Advances in comparative and environmental physiology*. Vol. 15. Springer, Berlin.
- Baum, D. A., and A. Larson. 1991. Adaptation reviewed: a phylogenetic methodology for studying character macroevolution. *Systematic Zoology* 40:1–18.
- Béani, L., and F. Dessi-Fulgheri. 1995. Mate choice in the grey partridge, *Perdix perdix*: role of physical and behavioural male traits. *Animal Behaviour* 49:347–356.
- Béani, L., G. Panzica, G. Briganti, P. Persichella, and F. Dessi-Fulgheri. 1995. Testosterone-induced changes of call structure, midbrain and syrinx anatomy in partridges. *Physiology and Behavior* 58:1149–1157.
- Becker, J. B., S. M. Breedlove, and D. Crews. 1992. *Behavioral endocrinology*. MIT Press, Cambridge, Mass.
- Beletsky, L. D., D. F. Gori, S. Freeman, and J. C. Wingfield. 1995. Testosterone and polygyny in birds. Pages 1–41 in D. M. Power, ed. *Current ornithology*. Plenum, New York.
- Brooks, D. R., and D. A. McLennan. 1991. *Phylogeny, ecology, and behavior: a research program in comparative biology*. University of Chicago Press, Chicago.
- Burley, N. T. 1986. Sexual selection for aesthetic traits in species with biparental care. *American Naturalist* 127: 415–445.
- Burley, N. T., and R. Symanski. 1998. "A task for the beautiful": latent aesthetic preferences for white crests in two species of Australian grassfinches. *American Naturalist* 152:792–802.
- Cawthorn, J. M., D. L. Morris, E. D. Ketterson, and V. Nolan, Jr. 1998. Influence of experimentally elevated testosterone on nest defence in dark-eyed juncos. *Animal Behaviour* 56:617–621.
- Chandler, C. R., E. D. Ketterson, V. Nolan, Jr., and C. Ziegenfus. 1994. Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Animal Behaviour* 47:1445–1455.
- Chandler, C. R., E. D. Ketterson, and V. Nolan Jr. 1997.

- Effects of testosterone on use of space by male dark-eyed juncos when their mates are fertile. *Animal Behaviour* 54:543–549.
- Cheverud, J. M., and E. J. Routman. 1995. Epistasis and its contribution to genetic variance components. *Genetics* 139:1455–1464.
- Clark, M. M., and B. G. Galef, Jr. 1995. Prenatal influences on reproductive life history stages. *Trends in Ecology & Evolution* 10:151–153.
- Clark, M. M., L. Tucker, and B. G. Galef, Jr. 1992. Stud males and dud males: intra-uterine position effects on the reproductive success of male gerbils. *Animal Behaviour* 43:215–221.
- Clark, M. M., D. Desousa, J. Vonk, and B. G. Galef. 1997. Parenting and potency: alternative routes to reproductive success in male Mongolian gerbils. *Animal Behaviour* 54:635–642.
- Coddington, J. A. 1988. Cladistic tests of adaptational hypotheses. *Cladistics* 4:3–22.
- . 1994. The roles of homology and convergence in studies of adaptation. Pages 53–78 in P. Eggleton and R. I. Vane-Wright, eds. *Phylogenetics and ecology*. Academic Press, London.
- Collis, K., and G. Borgia. 1992. Age-related effects of testosterone, plumage, and experience on aggression and social dominance in juvenile male satin birds (*Ptilonorhynchus violaceus*). *Auk* 109:422–434.
- Crawley, J. N., and R. Paylor. 1997. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and Behavior* 31:197–211.
- Crews, D., ed. 1987. *Psychobiology of reproductive behavior: an evolutionary perspective*. Prentice Hall, Upper Saddle River, N.J.
- Cunningham, D. L., and P. B. Siegel. 1978. Response to bidirectional and reverse selection for mating behavior in Japanese quail *Coturnix japonica*. *Behavioral Genetics* 8:387–397.
- Curio, E. 1973. Towards a methodology of teleonomy. *Experientia* 29:1045–1058.
- Dawkins, R. 1982. *The extended phenotype*. Oxford University Press, Oxford.
- Denver, R. J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *American Zoologist* 37:172–184.
- Dittami, J., H. Hoi, and G. Sageder. 1991. Parental investment and territorial/sexual behavior in male and female reed warblers: are they mutually exclusive? *Ethology* 88:249–255.
- Drea, C. M., M. L. Weldele, N. G. Forger, E. M. Coscia, L. G. Frank, P. Licht, and S. E. Glickman. 1998. Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 2. Effects of prenatal anti-androgens. *Journal of Reproduction and Fertility* 113:117–127.
- Dudley, S. A., and J. Schmitt. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* 147:445–465.
- Dufty, A. M., Jr. 1989. Testosterone and survival: a cost of aggressiveness? *Hormones and Behavior* 23:185–193.
- East, M. L., and H. Hofer. 1997. The peniform clitoris of female spotted hyaenas. *Trends in Ecology & Evolution* 12:401–402.
- East, M. L., H. Hofer, and W. Wickler. 1993. The erect “penis” is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyaenas. *Behavioral Ecology and Sociobiology* 33:355–370.
- Eibl-Eibesfeldt, I. 1970. *Ethology: the biology of behavior*. Holt, Rinehart, & Winston, New York.
- Emlen, D. J., and H. F. Nijhout. 1999. Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Journal of Insect Physiology* 45:45–53.
- Endler, J. A. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, N.J.
- . 1995. Multiple trait evolution and environmental gradients in guppies. *Trends in Ecology & Evolution* 10:22–29.
- Enstrom, D. A., E. D. Ketterson, and V. Nolan, Jr. 1997. Testosterone and mate choice in the dark-eyed junco. *Animal Behaviour* 54:1135–1146.
- Fahrbach, S. E. 1997. Regulation of polyethism in bees and wasps by juvenile hormone. *Advances in the Study of Behavior* 26:285–316.
- Falconer, D. S. 1989. *Introduction to quantitative genetics*. 3d ed. Wiley, New York.
- Feder, M. E. 1999. Engineering candidate genes in studies of adaptation: the heat-shock protein Hsp70 in *Drosophila melanogaster*. *American Naturalist* 154(suppl.):S55–S66.
- Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist* 139:603–622.
- Foster, N. G., L. G. Frank, S. M. Breedlove, and S. E. Glickman. 1996. Sexual dimorphism of perineal muscles and motoneurons in spotted hyenas. *Journal of Comparative Neurology* 375:333–343.
- Frank, L. G. 1997a. Evolution of genital masculinization: why do female hyaenas have such a large “penis.” *Trends in Ecology & Evolution* 12:59–62.
- . 1997b. Reply from L. G. Frank. *Trends in Ecology & Evolution* 12:402.
- Fusani, L., L. Béani, and F. Dessi-Fulgheri. 1994. Testosterone affects the acoustic structure of the male call in

- the grey partridge (*Perdix perdix*). *Behaviour* 128: 301–310.
- Fusani, L., L. Béani, C. Lupo, and F. Dessì-Fulgheri. 1997. Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour* 54:1013–1018.
- Garland, T., Jr. 1988. Genetic basis of activity metabolism. I. Inheritance of speed, stamina, and antipredator displays in the garter snake *Thamnophis sirtalis*. *Evolution* 42:335–350.
- Getz, L. L., B. McGuire, T. Pizzuto, J. E. Hofmann, and B. Frase. 1993. Social organization of the prairie vole (*Microtus ochrogaster*). *Journal of Mammalogy* 74: 44–58.
- Glickman, S. E., E. M. Coscia, L. G. Frank, P. Licht, M. L. Weldele, and C. M. Drea. 1998. Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 3. Effect of juvenile gonadectomy. *Journal of Reproduction and Fertility* 113:129–135.
- Gould, S. J. 1981. Hyena myths and realities. *Natural History* 90:16–24.
- . 1986. The egg-a-day barrier. *Natural History* 95: 16–24.
- Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist program. *Proceedings of the Royal Society of London B, Biological Sciences* 205:581–598.
- Gould, S. J., and E. S. Vrba. 1982. Exaptation—a missing term in the science of form. *Paleobiology* 8:4–15.
- Grafen, A. 1988. On the uses of data on lifetime reproductive success. Pages 454–471 in T. H. Clutton-Brock, ed. *Reproductive success*. University of Chicago Press, Chicago.
- Grober, M. S., S. H. Fox, C. Laughlin, and A. H. Bass. 1994. GnRH cell size and number in a teleost fish with two male reproductive morphs: sexual maturation, final sexual status and body size allometry. *Brain, Behavior, and Evolution* 43:61–78.
- Groothuis, T., and G. Meeuwissen. 1992. The influence of testosterone on the development and fixation of the form of displays in two age classes of young black-headed gulls. *Animal Behaviour* 43:189–208.
- Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? *Science (Washington, D.C.)* 213:384–387.
- Harding, C. F., M. J. Walters, D. Collado, and K. Sheridan. 1988. Hormonal specificity and activation of social behavior in male red-winged blackbirds. *Hormones and Behavior* 22:402–418.
- Harvey, P. H., and M. D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford University Press, Oxford.
- Hegner, R. E., and J. C. Wingfield. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* 104:462–469.
- Hews, D. K., and M. C. Moore. 1995. Influence of androgens on differentiation of secondary sex characters in tree lizards, *Urosaurus ornatus*. *General and Comparative Endocrinology* 97:86–102.
- . 1996. A critical period for the organization of alternative male phenotypes of tree lizards by exogenous testosterone? *Physiology and Behavior* 60:425–429.
- Hews, D. K., R. Knapp, and M. C. Moore. 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Hormones and Behavior* 28:96–115.
- Hill, J. A., D. A. Enstrom, E. D. Ketterson, V. Nolan, Jr., and C. Ziegenfus. 1999. Mate choice based on static versus dynamic secondary sexual traits in the dark-eyed junco. *Behavioral Ecology* 10:91–96.
- Hillgarth, N., and J. C. Wingfield. 1997. Parasite-mediated sexual selection: endocrine aspects. Pages 78–104 in D. H. Clayton and J. Moore, eds. *Host-parasite evolution: general principles and avian models*. Oxford University Press, Oxford.
- Hillgarth, N., M. Ramenofsky, and J. C. Wingfield. 1997. Testosterone and sexual selection. *Behavioral Ecology* 8: 108–109.
- Jannett, F. J. 1982. Nesting patterns of adult voles (*Microtus montanus*). *Journal of Mammalogy* 63:495–498.
- Johnsson, J. I., E. Jönsson, and B. T. Björnsson. 1996. Dominance, nutritional state, and growth hormone levels in rainbow trout (*Onchorhynchus mykiss*). *Hormones and Behavior* 30:13–21.
- Jönsson, E., J. I. Johnsson, and B. T. Björnsson. 1996. Growth hormone increases predation exposure of rainbow trout. *Proceedings of the Royal Society of London B, Biological Sciences* 263:647–651.
- Kast, T. L., E. D. Ketterson, and V. Nolan, Jr. 1998. Variation in sperm reserves according to season, stage of reproduction, and testosterone treatment in the dark-eyed junco. *Auk* 115:684–693.
- Ketterson, E. D., and V. Nolan, Jr. 1992. Hormones and life histories: an integrative approach. *American Naturalist* 140(suppl.):S33–S62.
- Ketterson, E. D., V. Nolan, Jr., C. Ziegenfus, D. P. Cullen, and M. J. Cawthorn. 1991a. Non-breeding season attributes of male dark-eyed juncos that acquired breeding territories in their first year. *Acta XXI Congressus Internationalis Ornithologici* 2:1229–1239.
- Ketterson, E. D., V. Nolan, Jr., L. Wolf, C. Ziegenfus, A. M. Dufty, Jr., G. F. Ball, and T. S. Johnsen. 1991b. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and

- body mass in dark-eyed juncos (*Junco hyemalis*). *Hormones and Behavior* 25:489–503.
- Ketterson, E. D., V. Nolan, Jr., L. Wolf, and C. Ziegenfus. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *American Naturalist* 140:980–999.
- Ketterson, E. D., V. Nolan, Jr., M. J. Cawthorn, P. G. Parker, and C. Ziegenfus. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* 138:70–86.
- Ketterson, E. D., P. G. Parker, S. A. Raouf, V. Nolan, Jr., C. Ziegenfus, and C. R. Chandler. 1997. The relative impact of extra-pair fertilizations on variation in male and female reproductive success in dark-eyed juncos (*Junco hyemalis*). Pages 81–101 in P. G. Parker and N. T. Burley, eds. *Avian reproductive tactics: female and male perspectives*. Ornithological Monographs, no. 49. Allen Press, Lawrence, Kans.
- Klein, S. L., J. E. Hairston, A. C. DeVries, and R. J. Nelson. 1997. Social environment and steroid hormones affect species and sex differences in immune function among voles. *Hormones and Behavior* 32:30–39.
- Klukowski, L., J. M. Cawthorn, E. D. Ketterson, and V. Nolan, Jr. 1997. Effects of experimentally elevated testosterone on plasma corticosterone and corticosteroid-binding globulin in dark-eyed juncos (*Junco hyemalis*). *General and Comparative Endocrinology* 108:141–151.
- Kruuk, H. 1972. *The spotted hyena*. University of Chicago Press, Chicago.
- Lake, P. E. 1981. Male genital organs. Pages 1–61 in A. S. King and J. McLelland, eds. *Form and function in birds*. Vol. 2. Academic Press, London.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Lauder, G. V., 1996. The argument from design. Pages 55–91 in M. R. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, Calif.
- Lederhendler, I. I. 1997. Using new genetics tools to advance the behavioral neurosciences beyond nature versus nurture. *Hormones and Behavior* 31:186–187.
- Licht, P., T. Hayes, P. Tsai, G. Cunha, H. Kim, M. Golbus, S. Hayward, M. C. Martin, R. B. Jaffe, and S. E. Glickman. 1998. Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 1. Urogenital morphology and placental androgen production during fetal life. *Journal of Reproduction and Fertility* 113:105–116.
- Lieberburg, I., and F. Nottebohm. 1979. High-affinity androgen binding proteins in syringeal tissues of songbirds. *General and Comparative Endocrinology* 37:286–293.
- Ligon, J. D., R. Thornhill, M. Zuk, and K. Johnson. 1990. Male-male competition, ornamentation and the role of testosterone in sexual selection in red jungle fowl. *Animal Behaviour* 40:367–373.
- Luine, V., F. Nottebohm, C. Harding, and B. S. McEwen. 1980. Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. *Brain Research* 192:89–107.
- Marler, C. A., and M. C. Moore. 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behavioral Ecology and Sociobiology* 23:21–26.
- . 1989. Time and energy costs of aggression in testosterone-implanted free-living male mountain spiny lizards (*Sceloporus jarrovi*). *Physiological Zoology* 62:1334–1350.
- . 1991. Supplementary feeding compensates for testosterone-induced costs of aggression in male mountain spiny lizards (*Sceloporus jarrovi*). *Animal Behaviour* 42:209–219.
- Marler, C. A., G. Walsburg, M. L. White, and M. C. Moore. 1996. Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology* 37:225–231.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Møller, A. P. 1994. *Sexual selection and the barn swallow*. Oxford University Press, Oxford.
- Møller, A. P., N. Saino, G. Taramino, P. Galeotti, and S. Ferrario. 1998. Paternity and multiple signaling: effects of a secondary sexual character and song on paternity in the barn swallow. *American Naturalist* 151:236–242.
- Moore, M. C. 1991. Application of organization-activation theory to alternative male reproductive strategies: a review. *Hormones and Behavior* 25:154–179.
- Nelson, R. J. 1997. The use of genetic “knockout” mice in behavioral endocrinology research. *Hormones and Behavior* 31:188–196.
- Nilsson, L. A. 1988. The evolution of flowers with deep corolla tubes. *Nature (London)* 334:147–149.
- Nolan, V., Jr., E. D. Ketterson, C. Ziegenfus, D. P. Cullen, and C. R. Chandler. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male dark-eyed juncos. *Condor* 94:364–370.
- Norris, D. O. 1997. *Vertebrate endocrinology*. 3d ed. Academic Press, San Diego, Calif.
- Orchinik, M., and B. S. McEwen. 1994. Rapid steroid actions in the brain: a critique of genomic and nongenomic mechanisms. Pages 77–108 in M. Wehling, ed. *Genomic and non-genomic effects of aldosterone*. CRC, Boca Raton, Fla.

- Oring, L. W., A. J. Fivizzani, and M. E. El Halawani. 1989. Testosterone-induced inhibition of incubation in the spotted sandpiper (*Actitis macularia* sic). *Hormones and Behavior* 23:412–423.
- Owens, I. P. F., and R. V. Short. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology & Evolution* 10:44–47.
- Peek, F. W. 1972. An experimental study of the territorial function of vocal and visual display in the male red-winged blackbird (*Agelaius phoeniceus*). *Animal Behaviour* 20:112–118.
- Pfennig, D. W. 1990. The adaptive significance of an evolutionarily cued developmental switch in an anuran tadpole. *Oecologia (Berlin)* 85:101–107.
- . 1992a. Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution* 46:1408–1420.
- . 1992b. Proximate and functional causes of polyphenism in an anuran tadpole. *Functional Ecology* 6:167–174.
- Price, D. K., and N. T. Burley. 1993. Constraints on the evolution of attractive traits: genetic (co)variance of zebra finch bill colour. *Heredity* 71:405–412.
- Raouf, S. A., P. G. Parker, E. D. Ketterson, V. Nolan, Jr., and C. Ziegenfus. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (Aves: *Junco hyemalis*). *Proceedings of the Royal Society of London B, Biological Sciences* 264:1599–1603.
- Reznick, D., and J. Travis. 1996. The empirical study of adaptation in natural populations. Pages 243–289 in M. R. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, Calif.
- Rissman, E. F., S. R. Wersinger, J. A. Taylor, and D. B. Lubahn. 1997. Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Hormones and Behavior* 31:232–243.
- Ros, A. F. H., T. G. G. Groothuis, and V. Apanius. 1997. The relation among gonadal steroids, immunocompetence, body mass, and behaviour in young black-headed gulls. *American Naturalist* 150:201–209.
- Rose, M. R., and G. V. Lauder. 1996. Post-spandrel adaptationism. Pages 1–8 in M. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, Calif.
- Rosenblatt, J. S. 1992. Hormone-behavior relations in the regulation of parental behavior. Pages 219–259 in J. B. Becker, S. M. Breedlove, and D. Crews, eds. *Behavioral endocrinology*. MIT Press, Cambridge, Mass.
- Runfeldt, S., and J. C. Wingfield. 1985. Experimentally prolonged sexual activity in female sparrows delays termination of reproductive activity in their untreated mates. *Animal Behaviour* 33:403–410.
- Saino, N., and A. P. Møller. 1995. Testosterone-induced depression of male parental behavior in the barn swallow: female compensation and effects on seasonal fitness. *Behavioral Ecology and Sociobiology* 36:151–157.
- Saino, N., A. P. Møller, and A. M. Bolzern. 1995. Testosterone effects on the immune system and parasite infections in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behavioral Ecology* 6:397–404.
- Saino, N., C. Primmer, H. Ellegren, and A. P. Møller. 1997. An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). *Evolution* 51:526–570.
- Salvador, A., J. P. Veiga, J. Martin, P. Lopez, M. Abelenda, and M. Puerta. 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasite infestation. *Behavioral Ecology* 7:145–150.
- Scanes, C. G. 1986. Pituitary gland. Pages 383–402 in P. D. Sturkie, ed. *Avian physiology*. Springer, New York.
- Schluter, D. 1988. The evolution of finch communities on islands and continents: Kenya vs. Galapagos. *Ecological Monographs* 58:229–249.
- Schmitt, J. 1999. Introduction: experimental approaches to testing adaptation. *American Naturalist* 154(suppl.): S1–S3.
- Schoech, S. J., E. D. Ketterson, and V. Nolan, Jr. 1999. Exogenous testosterone and the adrenocortical response in the dark-eyed junco, *Junco hyemalis*. *Auk* 116:64–72.
- Schwabl, H. 1993. Yolk as a source of maternal testosterone for developing birds. *Proceedings of the National Academy of Sciences of the USA* 90:11446–11450.
- . 1996a. Environment modifies testosterone levels of a female bird and its eggs. *Journal of Experimental Zoology* 276:157–163.
- . 1996b. Maternal testosterone in the avian egg enhances postnatal growth. *Comparative Biochemistry and Physiology* 114A:271–276.
- Schwabl, H., D. W. Mock, and J. A. Gieg. 1997. A hormonal mechanism of parental favouritism. *Nature (London)* 386:231.
- Sefton, A. E., and P. B. Siegel. 1975. Selection for mating ability in Japanese quail. *Poultry Science* 54:788–794.
- Shanbhag, B. A., and P. J. Sharp. 1996. Immunocytochemical localization of androgen receptor in the comb, uropygial gland, testis, and epididymis in the domestic chicken. *General and Comparative Endocrinology* 101:76–82.
- Sheldon, F. H., and L. A. Whittingham. 1997. Phylogeny in studies of bird ecology, behavior, and morphology. Pages 279–299 in D. P. Mindell, ed. *Avian molecular evolution and systematics*. Academic Press, San Diego, Calif.

- Siegel, P. B., and E. A. Dunnington. 1990. Behavioral genetics. Pages 877–895 in R. D. Crawford, ed. *Poultry breeding and genetics*. Elsevier Science, Amsterdam.
- Silverin, B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Animal Behaviour* 28: 906–912.
- Sinervo, B., and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. Pages 149–185 in M. R. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, Calif.
- Sinervo, B., and R. B. Huey. 1990. Allometric engineering: an experimental test of the causes of interpopulational differences in locomotor performance. *Science* (Washington, D.C.) 248:1106–1109.
- Sinervo, B., and P. Licht. 1991a. The physiological and hormonal control of clutch size, egg size, and egg shape in *Uta stansburiana*: constraints on the evolution of lizard life histories. *Journal of Experimental Zoology* 257:252–264.
- . 1991b. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science* (Washington, D.C.) 252:1300–1302.
- Sinervo, B., P. Doughty, R. B. Huey, and K. Zamudio. 1992. Allometric engineering: a causal analysis of natural selection on offspring size. *Science* (Washington, D.C.) 258:1927–1930.
- Siva-Jothy, M. T. 1995. “Immunocompetence”: conspicuous by its absence. *Trends in Ecology & Evolution* 10: 205–206.
- Smith, D. G. 1972. The role of the epaulets in the red-winged blackbird (*Agelaius phoeniceus*) social system. *Behaviour* 41:251–268.
- Stearns, S. C. 1989. Trade-offs in life-history evolution. *Functional Ecology* 3:259–268.
- . 1992. *The evolution of life histories*. Oxford University Press, Oxford.
- Swallow, J. G., T. Garland, Jr., P. A. Carter, and W.-Z. Zhan. 1998. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *Journal of Applied Physiology* 84:69–76.
- Titus, R. C., E. D. Ketterson, and V. Nolan, Jr. 1997. High testosterone prior to song crystallization inhibits singing behavior in captive yearling dark-eyed juncos, *Junco hyemalis*. *Hormones and Behavior* 32:133–140.
- Travis, J. 1989. The role of optimizing selection in natural populations. *Annual Review of Ecology and Systematics* 20:279–296.
- Trobec, R. J., and L. W. Oring. 1972. Effects of testosterone propionate implantation on lek behavior of sharp-tailed grouse. *American Midland Naturalist* 87:531–536.
- Van Tienderen, P. H., and A. Van Hinsberg. 1996. Phenotypic plasticity in growth habit in *Plantago lanceolata*: how tight is a suite of correlated traits? *Plant Species Biology* 11:87–96.
- Watson, A., and R. Parr. 1981. Hormone implants affecting territory size and aggressive and sexual behaviour in red grouse. *Ornis Scandinavica* 12:55–61.
- Weatherhead, P. J., K. J. Metz, G. F. Bennett, and R. E. Irwin. 1993. Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds. *Behavioral Ecology and Sociobiology* 33:13–23.
- West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20:249–278.
- . 1992a. Adaptation: current usages. Pages 13–18 in E. F. Keller and E. A. Lloyd, eds. *Keywords in evolutionary biology*. Harvard University Press, Cambridge, Mass.
- . 1992b. Behavior and evolution. Pages 37–75 in P. R. Grant and H. S. Horn, eds. *Molds, molecules and metazoa*. Princeton University Press, Princeton, N.J.
- Wickler, W. 1966. Ursprung und biologische Deutung des Genitalpräsentierens männlicher Primaten. *Zeitschrift für Tierpsychologie* 23:422–437.
- Wingfield, J. C. 1984. Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *Auk* 101:665–671.
- Wingfield, J. C., G. F. Ball, A. M. Dufty, Jr., R. E. Hegner, and M. Ramenofsky. 1987. Testosterone and aggression in birds. *American Scientist* 75:602–608.
- Wingfield, J. C., R. E. Hegner, A. M. Dufty, Jr., and G. F. Ball. 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist* 136:829–846.
- Wilson, D. S. 1997. Introduction: multilevel selection theory comes of age. *American Naturalist* 150(suppl.): S1–S4.
- Winslow, J. T., N. Hastings, S. C. Carter, D. R. Harbaugh, and T. R. Insel. 1993. A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature* (London) 365:545–547.
- Yalcinkaya, T. M., P. K. Siiteri, J.-L. Vigne, P. Licht, S. Pavgi, L. G. Frank, and S. E. Glickman. 1993. A mechanism for virilization of female spotted hyenas in utero. *Science* (Washington, D.C.) 260:1929–1931.
- Young, L. J., J. T. Winslow, Z. Wang, B. Gingrich, Q. Guo, M. M. Matzuk, and T. R. Insel. 1997. The use of genetic “knockout” mice in behavioral endocrinology research. *Hormones and Behavior* 31:221–231.
- Young, L. J., Z. Wang, and T. R. Insel. 1998. Neuroendocrine bases of monogamy. *Trends in Neuroscience* 21: 71–75.
- Zera, A. J., and C. Zhang. 1995. Evolutionary endocrinology of juvenile hormone esterase in *Gryllus assimilis*:

- direct and correlated responses to selection. *Genetics* 141:1125–1134.
- Zera, A. J., J. Sall, and K. Grudzinski. 1997. Flight muscle polymorphism in the cricket *Gryllus firmus*: muscle characteristics and their influence on the evolution of flightlessness. *Physiological Zoology* 70:519–529.
- 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*. *American Naturalist* 152:7–23.
- Zuk, M. 1990. Reproductive strategies and disease susceptibility: an evolutionary viewpoint. *Parasitology Today* 6:231–233.
- . 1996. Disease, endocrine-immune interactions, and sexual selection. *Ecology* 77:1037–1042.
- Zuk, M., K. Johnson, R. Thornhill, and J. D. Ligon. 1990. Parasites and male ornaments in free-ranging and captive red jungle fowl. *Behaviour* 114:232–248.
- Zuk, M., T. S. Johnsen, and T. Maclarty. 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proceedings of the Royal Society of London B, Biological Sciences* 260:205–210.